Impact of pine plantations on the form and mobility of nitrogen in soils of the eastern escarpment region of South Africa

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Frontispiece: Landscape photograph of Graskop sampling area of the eastern escarpment of South Africa illustrating the juxtaposition of forest plantation and natural grassland vegetation.

ABSTRACT

Recent research in the eastern escarpment area of South Africa has documented enhanced NO_3^- concentrations in soil solution and stream water resulting from afforestation. There has been much research in the Northern Hemisphere regarding the qualitative and quantitative causes and consequences of N saturation in forest ecosystems. In order to assess the significance of local observations of afforestation-induced NO_3^- enhancement in a global context, a study was conducted to evaluate the influence of forest plantations (*Pinus* spp.) on N form and mobility in soils in the eastern escarpment area of South Africa.

Twenty soil samples were taken, half in grassland and half in forest, in the Graskop and Kaapsehoop areas of the eastern escarpment. Forest samples were taken as composites of approximately five individual samples in each stand from the top 20-25 cm of soil, combining the partially decomposed organic litter layer with the subjacent upper mineral soil horizon. Grassland samples were taken, again as composites, consisting of the upper mineral soil horizon (an organic litter layer was for the most part absent). Approximately three-quarters of each sample was air-dried, and crushed to pass through a 2-mm sieve and stored for analysis. The remaining quarter of each sample was passed through a 2-mm sieve and refrigerated at about 4°C in order to maintain field-moist conditions and to inhibit microbial transformations. Refrigerated samples were used for KCl-extractable NO_3^- and NH_4^+ analyses and N mineralisation experiments. The remaining analyses were performed on the air-dried samples.

This study included two facets: physical and chemical characterisation of soil samples; and a series of laboratory experiments. The solid phase of the soils was analysed for organic carbon, total nitrogen and particle size distribution. The soil solution was investigated by making saturated paste extracts which were analysed for major ions, trace elements, electrical conductivity (EC) and pH. Extractable base cations, acidity and inorganic nitrogen (NH_4^+ and NO_3^-) were also analysed after extraction with NH_4OAc or KCl solutions. The laboratory experiments, intended to investigate the apparent differences in soil N transformations and mobility resulting from vegetation, consisted of both aerobic and anaerobic incubation to assess N mineralisation, a NO_3^- sorption experiment and a soil to extract ratio dilution experiment.

General chemical and physical assessment of the soil samples demonstrated a consistently highly leached, clay-poor, humus-rich, acidic nature of all the soils. The soil solution composition is dominated by Na^+, K^+ and Cl^- and is characterised by low ionic strength with a median EC of 229 μScm^{-1}. Divalent cations (Ca^{2+} and Mg^{2+}) dominate the exchangeable base cation suite, complementing the monovalent cation dominance in the soil solution. Exchangeable acidity is dominant relative to total exchangeable base cations and the soils are characterised by a median acid saturation of 82%. The soils are characterised by generally low effective cation exchange capacity (CEC_e), reflecting their highly leached status, which is significantly correlated to organic C content suggesting that the majority of exchange sites are associated with humic substances. The interpretation of Al speciation and solubility, although limited due to the exclusion of dissolved organic carbon (DOC) analysis and the analytical techniques employed, tentatively suggests organic matter buffering of Al particularly in the strongly acidic forest soils.

The danger was recognised of presupposing that observed differences in soils are solely a result of vegetation, rather than of compounded site differences. Consequently, following the characterisation of the soils, a cluster analysis was performed to assess the legitimacy of grouping samples according to vegetation. The cluster analysis identified thirteen samples, seven forest and six grassland, for which vegetation is a legitimate grouping variable. These thirteen samples were then used to assess the impact of afforestation on soil chemical properties.

Mann-Whitney U tests ($p < 0.10$) revealed a number of variables that successfully discriminate between the two vegetation types. The soil properties that discriminate between the vegetation types can be broadly grouped into four general categories including acidity status, organic matter status, nitrogen status and soil texture. Parameters related to acidity status included (median values for forest and grassland soils are given in parentheses): soil pH (4.68 and 4.98), extractable acidity (41.9 and 8.6 $mmol_c kg^{-1}$), acid saturation (86 and 80%) and ΔpH (i.e. pH (H_2O) – pH (KCl): 0.7 and 0.9). Variables related to organic matter status included organic carbon (6.3 and 2.8%), CEC_e (44.5 and 13.4 $mmol_c kg^{-1}$) and EC (201 and 278 μScm^{-1}). Nitrogen status variables included total N (0.33 and 0.14%), NO_3^-/I (defined as the soil solution concentration of NO_3^- relative to ionic strength: 0.06 and 0.02), extractable NO_3^- (0.27 and 0.15 mmol kg^{-1}), anaerobic mineralisation rate (0.34 and 0.16 mmol NH_4^+ kg $soil^{-1} day^{-1}$) and net nitrification rate (0.036 and 0.006 mmol NO_3^- kg $soil^{-1} day^{-1}$). The soil texture-related variables were clay content (4.2 and 2.1%) and sand

content (83.4 and 92.0%). A step-wise discriminant analysis was performed identifying the following variables which best discriminate between the two vegetation types (in decreasing order of efficiency): extractable acidity, soil solution Ca^{2+}, ΔpH, extractable NO_3^-, soil solution NO_3^-/I, extractable Na^+ and rate of net nitrification.

The anaerobic mineralisation experiment revealed an enhanced rate of mineralisation (mmol NH_4^+ kg^{-1} day^{-1}) in the forest soils relative to those from grassland ($p = 0.04$). This enhancement in the forest samples was attributed to the enhanced total N content in the forest samples relative to the grassland samples; the correlation between rate of mineralisation and total N is characterised by $r_s = 0.94$ ($p < 0.001$). Expressing the rate of mineralisation relative to the organic carbon content (mmol NH_4^+ $kgOC^{-1}$ day^{-1}) eliminates the difference in rate between the vegetation types ($p = 0.57$), suggesting that although the total amount of N mineralised per kg of soil is considerably larger in the forest samples, the fraction of total N that is mineralisable is virtually the same in soils from the two vegetation types.

The aerobic mineralisation experiment revealed an enhanced rate of net nitrification in the forest samples relative to the grassland samples. The rate of net nitrification neither correlated to organic matter status nor to total N status. There is, however, a slight negative correlation between rate of net nitrification and C/N ratio ($r_s = -0.42$, $p = 0.16$) and the significance of the correlation is improved for the forest samples alone ($r_s = -0.68$, $p = 0.09$, $n = 7$). There also appears to be a slight inhibiting effect of decreasing soil pH on rate of net nitrification, although not highly significant ($r_s = -0.48$, $p = 0.09$). There does not appear to be a soil chemical property that has been measured that can fully account for the enhanced rate of net nitrification in the forest samples relative to the grassland samples. It is possible that there are differences in soil microbiological and/or biochemical properties, particularly with respect to the nitrifying communities, between the vegetation types contributing to the enhanced rate of net nitrification in the forest samples. The enhanced rate in the forest samples does, however, seem to account for the enhanced soil solution NO_3^-/I ($r_s = 0.68$, $p < 0.01$) and extractable NO_3^- ($r_s = 0.69$, $p < 0.01$) status in the forest samples relative to the grassland samples.

In general, the conversion of organic N to NH_4^+ appears to be related to total N content and consequently, to organic matter content. The proportion of total N that is mineralisable is, however, virtually the same between vegetation types. Enhanced nitrification in the forest samples, on the other hand, is not correlated to enhanced organic matter content. Both

ammonification and nitrification are slightly negatively correlated to C/N ratio (particularly within the forest samples). There is, however, no difference in C/N ratio between vegetation types and, therefore, C/N ratio does not fully explain the apparently enhanced mineralisation rates in the forest samples relative to the grassland samples. Varying C/N ratio may, however, explain differences in rates of mineralisation within the forest samples.

The results of this study emphasise the importance of organic matter accumulation in the forest floor coupled with C/N ratio in influencing N mineralisation and nitrification and resulting in an enhanced NO_3^- status in forest soils. Further studies are necessary, including field measurements during different seasons, to confirm these findings and to gain a better understanding of the relative contributions of enhanced interception of the forest canopy of atmospheric N and of microbiological activity in influencing overall N status.

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INTRODUCTION

Elevated inputs of atmospheric nitrogen, derived from both industry and agriculture, have been a cause for concern in relation to the more sensitive forest ecosystems in Europe and North America. Nitrogen saturation, qualitatively defined as the availability of ammonium and nitrate exceeding total plant and microbial nutrient demand (Aber *et al.*, 1989), is purported to have serious environmental consequences such as acidification and eutrophication of soil and water (Emmett *et al.*, 1993) and also forest decline (Roelofs *et al.*, 1985). Enhanced interception and evaporative concentration of atmospheric inorganic nitrogen by forest canopies accounts for increased inputs of nitrogen to forest soils relative to non-forested areas (Emmett and Reynolds, 1996). Emmett *et al.* (1993) reported on the various stages associated with the progression towards nitrogen saturation of forests along an age sequence of spruce stands in northern and central Wales. The conversion of grassland to forest results in an increased nitrogen mineralisation, presumably resulting from drying of soil due to enhanced evapotranspiration and corresponding to the accumulation of biomass. This available nitrogen is retained while the stand is young, but becomes superfluous as a stand reaches maturity. Emmett *et al.* (1993) reported that no stimulation of net mineralisation or nitrification in the soil is required before elevated nitrate losses are observed in mature stands. Rather the explanation lies in changes in capacity due to stand age, such that inorganic nitrogen losses in mature stands correlate to atmospheric inputs, whereas prior to maturity, tree uptake appears to be the most important factor controlling inorganic nitrogen losses.

Research in Southern Africa suggests that afforestation with *Pinus* species has a significant impact on soil and stream water chemistry draining forest catchments (Morris, 1986; du Toit, 1993; Nowicki, 1997; Sugarman, 1999). Afforestation reportedly induces acidification, reducing acid neutralising capacity and base saturation in sensitive upland soils in South Africa. Afforestation in the eastern escarpment area of South Africa also appears to coincide with enhanced nitrate concentrations in soil solutions and stream waters draining forest catchments (Fey and Netch, 1994; Nowicki, 1997; Fey *et al.*, 1999). Fey *et al.* (1999), for example, reported an average four-fold enhancement of nitrate concentration in stream waters draining forest catchments relative to comparable stream waters draining grassland catchments. Similarly, Nowicki (1997) reported a dramatic afforestation-induced enhancement of nitrate in soil solutions, such that in some cases nitrate becomes the dominant soluble anion. This observed afforestation-induced enhancement of nitrate status, in conjunction with the current research regarding nitrogen saturation in forest ecosystems in the

Northern Hemisphere (e.g. Aber *et al.*, 1989 and Gundersen *et al.*, 1998), demands further study of afforestation-induced changes in soil chemical properties with special reference to soil nitrogen form and mobility.

The key question motivating this study is why are there apparent differences in soil nitrogen form and mobility between forest and grassland soils in the eastern escarpment area of South Africa? In order to address this question a study, culminating in this thesis, was proposed with the following primary objectives. Firstly, to assess and quantify observed differences in soil nitrogen form and mobility between grassland and forest soils. Secondly, to examine major processes associated with organic and inorganic nitrogen that may elucidate causes of the observed differences in nitrogen status between the two vegetation types. Thirdly, to assess broad soil chemical properties affected by afforestation and attempt to correlate afforestation-induced chemical changes with observed changes in nitrogen form and mobility. In order to meet the aforementioned objectives twenty soil samples were taken, half in grassland and half in forest, from the Graskop and Kaapsehoop areas of South Africa. The samples were characterised chemically and a series of laboratory experiments regarding nitrogen form and mobility was conducted.

The first phase of this study included a literature review (Chapter 1) relating to the major transformations of nitrogen in the soil environment. The second chapter reports on the characterisation of the soils with special reference to nitrogen status. Chapter 2 also details the sampling strategy (collection and preparation), study areas and statistical treatment of data. Following the general characterisation of the soils, a series of experiments were conducted including aerobic and anaerobic nitrogen mineralisation, nitrate sorption and a soil to extract ratio dilution experiment, the results of which are presented in Chapter 3. The final chapter of this thesis consists of a general discussion and conclusions with special emphasis on the integration of the major findings of this study with current literature and previous studies assessing afforestation-induced chemical changes in the eastern escarpment area of South Africa.

Chapter 1. Nitrogen transformations in soils: a review

1.1 Introduction

High inputs of anthropogenic N have caused concern for highly sensitive forest ecosystems in much of Europe and North America. Nitrogen saturation, originally defined as the availability of NH_4^+ and NO_3^- exceeding total plant and microbial nutrient demand (Aber *et al.*, 1989), is purported to have serious environmental consequences such as soil and water acidification and eutrophication as well as nutrient imbalances in forest ecosystems (Gundersen *et al.*, 1998). Despite elevated atmospheric inputs, N is still commonly the growth-limiting nutrient in forest ecosystems around the world (Van Miegroet *et al.*, 1990). As anthropogenic N inputs increase to levels as high as 50-60 kg N $ha^{-1}yr^{-1}$ in parts of Western Europe (Bredemeier *et al.*, 1998), N-limited ecosystems are increasingly becoming N-saturated.

The aim of this chapter is to review the major N transformations in soils. Particular attention will be given to factors affecting net mineralisation of N and the possibility of mineral N-saturation in ecosystems. Possible negative consequences of excess mineral N in soil ecosystems will also be examined.

1.2 Nitrogen transformations

There are a number of N transformations in soils. Ideally N cycles exist in an equilibrium that is dictated by the biotic, abiotic and soil properties of the ecosystem. A brief description of the major nitrogen transformations in soils is presented in Table 1.1 and their position in the overall N cycle is depicted in Figure 1.1. This section will defer discussion of the factors which affect these major transformations to a later part of the review.

1.2.1 Nitrogen fixation

Nitrogen fixation is the biologically mediated process by which inert atmospheric N_2 is reduced to organically bound N. Nitrogen fixation is the most important natural process that

increases N content in soils (Wild, 1988). Nitrogen can be fixed by micro-organisms that are free-living or by those living symbiotically with plants (Tisdale *et al.*, 1985).

Table 1.1 A brief description of major nitrogen transformations in soils

Nitrogen fixation	Reduction of dinitrogen to organically bound nitrogen $N_2 \rightarrow$ org-N
Ammonification	Conversion of organic nitrogen to inorganic ammonium org-N $\rightarrow NH_4^+$
Nitrification	Oxidation of ammonium to nitrite and nitrate $NH_4^+ \rightarrow NO_2^- \rightarrow NO_3^-$
Immobilisation	Assimilation of inorganic nitrogen into cell biomass by living organisms $NH_4^+/NO_3^-/NO_2^- \rightarrow$ org-N
Denitrification	Reduction of nitrate to nitrous oxide and dinitrogen $NO_3^- \rightarrow N_2O \rightarrow N_2$

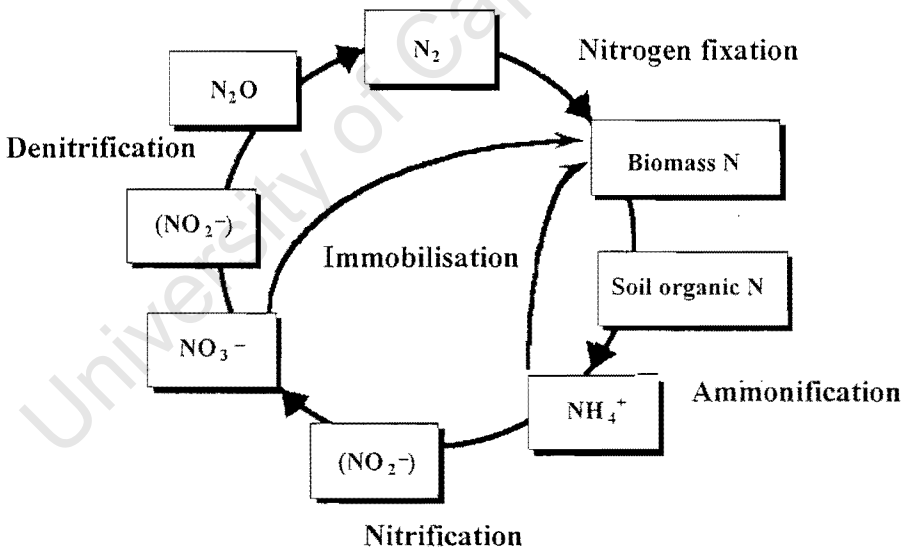


Figure 1.1 A schematic depiction of the major soil N transformations and their position within the overall N cycle.

Despite the wide range of micro-organisms associated with N fixation, the chemistry mediating the reduction is similar for all. All biologically mediated fixation consists of similar mechanisms using a nitrogenase complex consisting of dinitrogenase, a Mo- and Fe-coated protein and dinitrogenase reductase, an O_2 sensitive Fe protein (Tisdale *et al.*, 1985).

Certain N fixing organisms rely on the products of photosynthesis as an energy source; sunlight is, therefore, often a limiting factor. High levels of inorganic N, particularly NH_4^+, leading to biological assimilation are also known to inhibit N fixation. Aeration is also a crucial environmental factor influencing N fixation, as dinitrogenase reductase is O_2 sensitive (with a half-life in air of less than two minutes). The oxidation states and speciation of Mo and Fe, which are essential to the production of dinitrogenase, are also affected by aeration (Harris, 1988).

Nitrogen fixation is associated with certain ecosystems and vegetation, specifically with symbiotic N fixers as well as free-living N fixers. Soils beneath N-fixing vegetation typically have larger total N and available N than other soils. This increased N pool may lead to faster rates of turnover throughout the N cycle. Binkley *et al.* (1992) reported that soils under N-fixing alder had net N mineralisation rates of 9 compared to 0-2.1 kmol $ha^{-1}year^{-1}$ in soils under conifers.

1.2.2 Ammonification

Ammonification is the decomposition of organic N, usually amine groups, to NH_4^+. Heterotrophic micro-organisms decompose organic matter as a source of C and N to meet nutritional needs. The final product of the decomposition of proteins is NH_4^+, which will be used by the organisms for cell synthesis. If there is an excess of N in the organic substrate, the organisms will excrete NH_4^+ as a waste product, resulting in ammonification. Alternatively, if there is insufficient N in the decomposing organic matter to meet the heterotrophs' nutritional needs, they will utilise mineral N from the soil, resulting in N immobilisation (Harris, 1988).

The delicate balance between ammonification and immobilisation is mediated by the C/N ratio of the decomposing matter and the cells being synthesised. C/N ratios vary a great deal between organic substances, for example, C/N for some animal waste has been calculated as approximately 5, compared to values of 100 for cereal straw. The optimal C/N ratio at which neither ammonification nor immobilisation occurs is roughly between 20-30 (Tisdale *et al.*, 1985; Harris, 1988; Gundersen, 1998). There are, however, a number of other factors that will affect decomposition of organic matter and the balance between ammonification and immobilisation. Table 1.2 lists the C/N ratios for a variety of organic substances.

Table 1.2 C/N ratios in a selection of organic materials (after Tisdale *et al.*, 1985, Table 5-3, p.122.)

Organic substance	C/N ratio	Organic substance	C/N ratio
Sweet clover (young)	12:1	Shale oils	124:1
Barnyard manure (rotted)	20:1	Oak	200:1
Clover residues	23:1	Pine	286:1
Green rye	36:1	Crude oil	388:1
Corn stover	60:1	Sawdust	400:1
Grain straw	80:1	Spruce	1000:1
Timothy	80:1	Fir	1257:1

1.2.3 Nitrification

Nitrification is the biologically mediated oxidation of NH_4^+ to NO_3^- via NO_2^-. Organisms capable of nitrifying NH_4^+ in soils are numerous and diverse. The oxidation of NH_4^+ to NO_2^- is the first step in nitrification and is mostly achieved by a genus of obligate autotrophic bacteria known as *Nitrosomonas*. There is, however, evidence that there are several heterotrophic organisms (including bacteria, actinomycetes and fungi) which are capable of converting reduced N to NO_2^-. The further oxidation of NO_2^- to NO_3^- is largely controlled by a second genus of obligate autotrophic bacteria, *Nitrobacter*. Although *Nitrobacter* dominate this conversion, some heterotrophs are also capable of producing NO_3^- (Tisdale *et al.*, 1985). The chemical equations of the two step nitrification can be expressed as follows (Harris, 1988):

Nitrosomonas stage: $2NH_4^+ + 3O_2 \rightarrow 2NO_2^- + 2H_2O + 4H^+ + 12e^-$ (1)

Nitrobacter stage: $2NO_2^- + O_2 \rightarrow 2NO_3^- + 4e^-$ (2)

1.2.4 Immobilisation

Mineralisation consists of NH_4^+ and NO_3^- production via ammonification and nitrification, respectively. Immobilisation can be viewed as the opposite of mineralisation; i.e. inorganic N is incorporated into cell biomass in order to meet microbial nutrient needs. Immobilisation of inorganic N is likely to occur when low-N organic matter begins decomposing in soils. The

balance between immobilisation and mineralisation is strongly influenced by the C/N ratio of the decomposing matter and the cells being synthesised. Figure 1.2 shows an example of immobilisation of added NO_3-N in the presence of rapidly decomposing organic matter. High CO_2 evolution indicates a period of active decomposition, which corresponds to high immobilisation levels. As decomposition levels off, as indicated by a sharp decrease in CO_2 evolution, N immobilisation is impeded and mineralisation begins to occur.

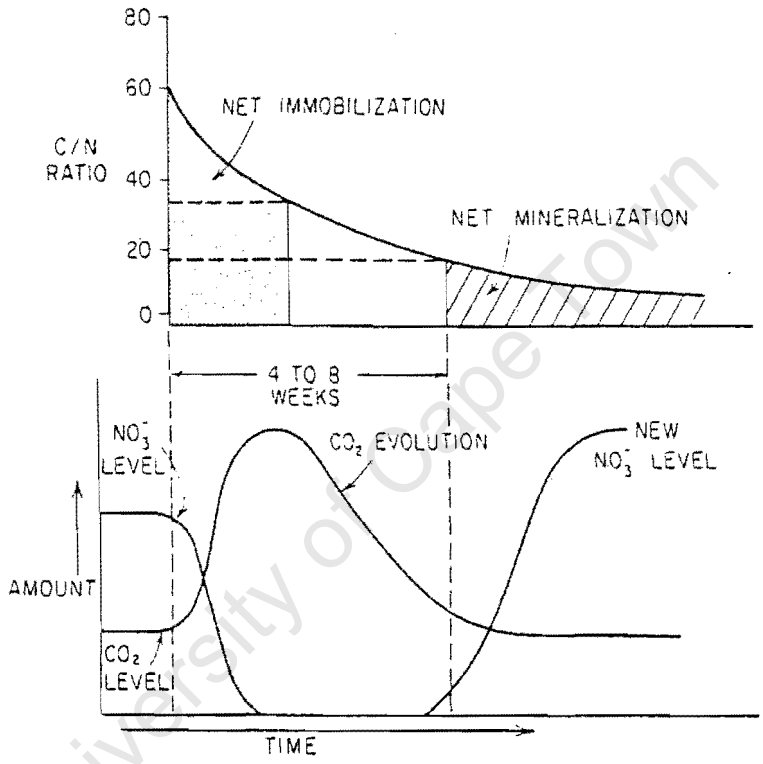


Figure 1.2 Changes in nitrate levels of soil during the decomposition of low-nitrogen crop residues (after Tisdale *et al.*, 1985, p. 124).

1.2.5 Denitrification

Microbial denitrification is the reduction of NO_3^- producing primarily gaseous N_2O and N_2. There are a number of groups of bacteria that are responsible for denitrification, the majority of which are facultative, functioning under aerobic and anaerobic conditions. The schematic sequence of reduction reactions comprising denitrification are as follows (Harris, 1988):

$$NO_3^- \rightarrow NO_2^- \rightarrow (NO) \rightarrow N_2O \rightarrow N_2 \qquad (3)$$

Denitrification occurs under low O_2 conditions, in the presence of NO_3^-, organic substrates and the appropriate denitrifying communities. Due to the variety of factors affecting denitrification, it clearly does not occur throughout the soil profile in a consistent manner (Wild, 1988).

1.3 Factors affecting net N mineralisation in soil ecosystems

Net N mineralisation refers to the net amount of mineral N produced (or consumed) over time, and represents the amount mineralised plus the amount deposited minus the amount immobilised, denitrified, taken up by vegetation, volatilised, and leached from the soil profile. It is clear that there are numerous factors that will affect net mineralisation rates in soil. These factors include soil properties, biotic properties (vegetation and microbial populations) and environmental factors. The following discussion will focus on soil properties and environmental factors that influence net N mineralisation in soils.

1.3.1 Temperature

Nitrogen mineralisation, as previously described, consists of biologically mediated processes. It is, therefore, not surprising that temperature plays a significant role in the regulation of these processes. It has been established that the dependence of mineralisation on temperature is characterised with a coefficient, Q_{10}, equal to two between 0-35°C. That is, for every 10°C increase in temperature across this range, mineralisation rate is expected to double (Tisdale *et al.*, 1985). However, Rice and Havlin (1994) reported that Q_{10} values range from 2-2.75 over different climatic zones and soil textures, with sandy soils having higher values than loams. The graph in Figure 1.3 shows the relationship between mineralisation and temperature with a Q_{10} of two.

Fluctuating temperatures are a reality in any soil environment and may affect mineralisation. Rice and Havlin (1994) assert that nitrification is more sensitive to temperature fluctuations than ammonification. Since ammonification is generally the rate-limiting step in N mineralisation, it is assumed that N mineralisation will not be greatly affected by moderate fluctuating soil temperatures.

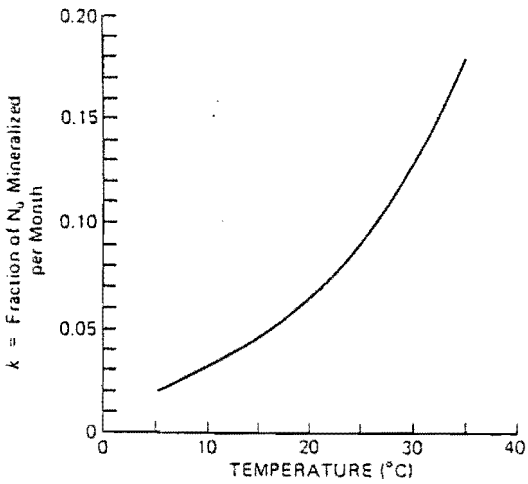


Figure 1.3 Fraction of N mineralised per month in relation to temperature (after Tisdale *et al.*, 1985, Figure 5-9, p. 131)

Similar to nitrification, denitrification is reported as very sensitive to temperature. Harris (1988) reports that NO_3^- loss can double with a temperature increase of 10°C over the range of 10°C to 35°C, while between 0°C and 5°C denitrification is much reduced. Tisdale *et al.* (1985) report further that denitrification will proceed at slightly higher rates when temperature is increased in the range of 25°C and 60°C.

1.3.2 Soil water content and aeration

Soil water content substantially influences N mineralisation, both by itself and due to its effect on aeration. Rice and Havlin (1994) report an optimal water-filled pore space of ~60% for aerobic microbial activity. Both maximum mineralisation and immobilisation should occur at this water content. At low moisture levels microbial activity is impaired and ammonification will be restricted, limiting the amount of NH_4^+ available for nitrification (Harris, 1988). As moisture content increases, mineralisation is affected as aeration becomes limiting. Anaerobic activity occurs at much slower rates than aerobic activity. Under anaerobic conditions, microbial N requirements are reduced, leading to a build-up of mineral NH_4^+. Nitrification is an oxidative process that does not occur at redox potentials less than +200mV (Harris, 1988). Poorly aerated soils are not expected to nitrify.

According to Tisdale *et al.* (1985), a strong interactive effect of soil moisture and temperature exists. As discussed in the previous section, mineralisation increases as temperature increases

from 15 to 30°C. At below optimum soil moisture contents, it has been reported that net N mineralisation increases at 30°C to levels higher than expected had there been just an additive relationship between the two variables. Tisdale *et al.* (1985) advise that these variables should not be considered independently as doing so will discount synergistic effects in treatments.

1.3.3 C/N ratio

The importance of the C/N ratio of organic matter on net mineralisation has already been touched upon in conjunction with the balance between ammonification and immobilisation. If decomposing organic matter has N in excess of the nutrient demands of the decomposers, mineral N (NH_4^+) will be excreted. Alternatively, if the organic matter is deficient in N, mineral N (NH_4^+, NO_3^-) will be assimilated into the soil organic matter. Problems arise when plant residue or soil amendments with high C/N ratios (Table 1.2) begin to decompose in soils. The large energy supply results in a microbial population explosion, which will need to rely on mineral N to meet its nutritional needs. It is possible that plant nutrition will suffer as a consequence of competition between microbes and vegetation for mineral N (Tisdale *et al.*, 1985). On the other hand, as the N content in the soil organic matter increases, mineralisation increases, resulting in the production of mineral N and ultimately N-saturation.

It has been theorised that additional N (fertiliser or atmospheric deposition) will be readily absorbed in N-limited ecosystems through enhanced plant growth and accelerated internal cycling of N. As the system becomes N-saturated as opposed to N-limited, however, NO_3^- will start to leach out of the system and impaired nutrition will appear as an integrated response to changes in processes of the N-cycle (Gundersen, 1998). Gundersen's (1998) research in conjunction with the NITREX[1] project showed that a N-limited spruce forest at Klosterhede, Denmark did retain most of the added N (35 kg N $ha^{-1}yr^{-1}$ with ambient N deposition of 15-20 kg N $ha^{-1}yr^{-1}$). Nitrogen leaching did, however, occur during the experiment because of hydrological transport of mobile NO_3^- which had been input directly by deposition. The implication is that even N-limited ecosystems may experience episodic NO_3^- leaching (usually taken as an indicator of N saturation) due to enhanced N deposition.

[1] The NITREX (NITRogen saturation EXperiments) consists of ecosystem-scale experiments designed to quantify the rates and timing of ecosystem responses to increases in N deposition (Gundersen *et al.*, 1998)

Research conducted by Emmett *et al.* (1998), also through the NITREX project, suggests that a change in rate of nitrification is a consequence of changes in status of N pools rather than present day deposition inputs. The authors concluded that a decrease in C/N ratio in the forest floor necessarily pre-empts the stimulation of nitrification at which time losses due to NO_3^- leaching may exceed inputs. Although a system may be technically N-saturated, such that mineral N exceeds plant and microbial N demands, additional N will not stimulate mineralisation unless the N pool is large enough. Nitrogen inputs may be leached out directly from the system or retained at exchange sites. This conclusion is consistent with the positive and highly significant relationship between C/N ratio and nitrification rate found throughout the NITREX project (Gundersen *et al.*, 1998).

1.3.4 Salinity

Salinity affects mineralisation, namely nitrification, through two distinct paths. Firstly, changes in osmotic potential of soil solutions influence the rates of nitrification, and secondly, nitrifying micro-organisms can be directly affected due to specific toxicity. Roseberg *et al.* (1986) studied the effects of Cl^- and osmotic potential on nitrification. Their results indicated that a decrease in osmotic potential associated with the addition of salt amendments inhibits nitrification. It was also noted that a decrease in osmotic potential coupled with specific Cl^- toxicity had a larger inhibiting effect on nitrification, indicating that both variables (osmotic potential and Cl^- concentration) contribute to the inhibition of nitrification.

Harris (1988) suggests that it may be possible for nitrifiers to adapt to salinity to some extent, but not to the extreme levels found in saline alkaline soils. This suggestion stems from the fact that most studies of salinization effects on nitrification are conducted by adding salts to non-saline soils without an adjustment period.

1.3.5 Acidity and pH

It has been reported that nitrification does not take place readily in very acid soils, with an optimum pH reported as 5.5 - 8.0 (Harris, 1988 and Tisdale *et al.*, 1985). However, there is a copious amount of recent work suggesting that this is not true. A field study conducted by van Breemen *et al.* (1987) consisting of four plots, three acidic (pH 3-4) and the fourth calcareous (pH 6.5-7.5), suggests that substantial nitrification occurs both in highly acidic and

calcareous soils. The authors report that the acidity formed during N transformations (3-9 $kmol_c ha^{-1} yr^{-1}$) accounted for a major part of all the soil acidification taking place. Further work conducted by Tietema *et al.* (1992) considered the nature and pH-dependence of NO_3^- production in N-saturated forest soils. An incubation experiment was carried out using soil cores from five acid Dutch soils and four of the five were found to nitrify substantially. The nitrification that occurred was acid-tolerant. The authors emphasised that this type of nitrification is of utmost importance in N-saturated acid forest soils.

Biederbeck *et al.* (1996) studied the effects of ten years of urea and ammonia fertilisation on microbial populations and soil biochemical properties. The soil pH was found to decrease proportionally to the rate at which N was added and after ten years pH ($CaCl_2$) values ranged between 4.32-5.42. Despite the acidifying effect N fertilisation had on the soil, the nitrifying bacteria seemed to respond positively to the rate of N application. The authors suggested that since the soils were naturally acidic the native bacterial population may have already been adapted to acidic conditions and was, therefore, able to thrive in the acidic conditions generated by fertilisation.

Pennington and Ellis (1993) evaluated the extent to which autotrophic and heterotrophic nitrifiers were active in fresh and incubated acid soils. The authors concluded that nitrification occurred readily at pH as low as 3.4 (only below pH 3.4 was there a decrease in rate of nitrification) and it was acidophyllic autotrophs that were responsible for the majority of nitrification in those soils. Research conducted by De Boer *et al.* (1995) resulted in the successful adaptation of the acid-sensitive, ammonium-oxidising bacterium *Nitrosospira* strain AHB1 to oxidise ammonium at pH 4. Adaptation was obtained with two procedures, firstly, by immobilising the bacteria in alginate beads and secondly, by exposing them to pH fluctuations. Prior to the discovery of these two adaptation methods, the bacteria had been isolated from acid soils, but their lower pH limit for nitrifying in pure cultures was much higher than the lower limit that existed in the soils. Acid-tolerant chemolithotrophic bacteria have been reported to account for nitrification in acid forest, acid heathland and N-fertilised acid tea soils (De Boer *et al.*, 1995).

Current research suggests that nitrification is often not acid limited, particularly in environments that are naturally acidic prior to further acidification. It is, therefore, not justified to say that nitrification is always self-limiting due to the acidity produced, i.e. every

mole of NH_4^+ oxidised to NO_3^- produces two moles of acidity as protons (equation 1), and therefore, nitrification can play a crucial role in long-term acidification in soils.

1.3.6 Mineralogy and texture

Interactions between soil mineralogy and texture have also been shown to influence N mineralisation through physical and chemical protection of organic matter from decomposition. It has been demonstrated that increasing clay content increases organic C stability and results in a decrease in net N mineralisation (Hassink *et al.*, 1993; Motavalli *et al.*, 1995).

Montmorillonitic and smectitic clays exhibit a greater organic C stabilisation than kaolinitic clays. This greater stabilisation has been attributed to physical protection of organic C in interlayer clay spaces (Ladd *et al.*, 1992). Allophanic soils have also been found to stabilise organic C through complexation with Fe and Al oxides, resulting in a decrease in N mineralisation (Motavalli *et al.*, 1995).

Ammonium ions, similarly to K^+, can become fixed within clay mineral interlayer spaces. Soils that contain clay minerals such as hydrous mica and vermiculite can fix NH_4^+ and, therefore, decrease the availability of nitrifiable substrate inhibiting nitrification (Harris, 1988).

1.4 The effects of excess mineral nitrogen in soils (nitrogen saturation)

1.4.1 Contamination of surface and groundwater

One of the most serious consequences of excess mineral N in soils is N leaching and contamination of nearby surface and groundwater. Nitrate leaching is of particular concern compared to NH_4^+ since NO_3^- is a strong acid anion and it has an enhanced mobility and will be transported through soil without being retained significantly (McBride, 1994). Ammonium, on the other hand, will be retained to a certain extent on cation exchange surfaces in the mineral soil. The degree to which NH_4^+ is retained on exchange surfaces depends on the concentration of NH_4^+ and its selectivity for the exchange surfaces. As mentioned earlier, NH_4^+ has the same charge as K^+ and a similar ionic radius and, therefore,

behaves similarly in cation exchange reactions. Due to the ideal size of the two cations, they are selectively associated with vermiculite and micaceous minerals (McBride, 1994). Despite NO_3^- mobility, it is expected to interact with anion exchange sites associated with variable charge oxides. However, NO_3^- is considered an "indifferent anion" as it adsorbs passively on exchange surfaces and will readily be exchanged for other anions (McBride, 1994). Excess NO_3^-, therefore, often leaches out of soils despite attenuation due to anion exchange surfaces.

It has been shown that NO_3^- leaching can occur substantially before N-saturation has been reached. Gundersen (1998) reported that hydrological transport of input NO_3^- was responsible for immediate leaching of the anion despite N-limited conditions. Emmett *et al.* (1998) reported that under ambient N deposition (16.1 kg N $ha^{-1}yr^{-1}$) there is significant NO_3^- leaching (8 kg N $ha^{-1}yr^{-1}$) in a 30 year-old Sitka spruce stand in the Aber forest. Treatment with $NaNO_3$ and NH_4NO_3 at the Aber site revealed that the N associated with the oxidised anion was readily leached whereas the NH_4^+ was retained in the system. The authors concluded that any future increases in N deposition in the oxidised form would result in immediate increases in NO_3^- leaching, which could lead to serious eutrophication and acidification in stream water.

Nitrate contamination of drinking water is a concern as it can be reduced to NO_2^- in the gastrointestinal tract. Once in the system, NO_2^- combines with haemoglobin to form methaemoglobin, which is unable to serve as an O_2 carrier. This condition, methaemoglobinaemia, is of particular hazard to infants under three months of age (DWAF, 1993). The DWAF guideline for NO_3^- in drinking water is 6 mg/l, whereas elevated concentrations of NO_3^- in stream waters draining forest catchments characterised by N-saturation are of the order of 0.6 mg/l (Emmett *et al.*, 1993), suggesting that there is little threat of NO_3^- contamination associated with N-saturated forest ecosystems.

1.4.2 Long-term acidification

Long-term acidification of soils and waters is associated with nitrification and excess mineral N in soils. As seen in equation 1, each mole of NH_4^+ oxidised produces two moles of acidity. Recent research suggests that nitrification is not always acid limited, and will continue to produce acidity if the NH_4^+ substrate is available. If the NO_3^- produced were utilised by plants, acidification would not be long-term since OH^- or HCO_3^- is exuded by NO_3^- feeding

plants (McBride, 1994). Long-term acidification is an indirect consequence of NO_3^- leaching. Due to electroneutrality, the anions leached from a system must be balanced by the equivalent amount of cations. Nitrification produces NO_3^- anions and H^+ cations, some protons will replace base cations that are subsequently leached with the NO_3^-. The removal of base cations and the subsequent leaching of H^+ and Al^{3+} poses a serious threat of long-term acidification in the soil and nearby ground and surface water. As the pH of the soil and water decreases, a wide spectrum of metals will increasingly become more soluble and mobile, which can lead to detrimental toxicity to vegetation, aquatic life and human health.

At the Aber forest site, Emmett *et al.* (1998) reported that five years of enhanced N deposition (35 kg N $ha^{-1}yr^{-1}$) as NH_4NO_3 and $NaNO_3$ lead to steady acidification and substantial mobilisation of Al in nearby stream water. Bredemeier *et al.* (1998) reported Al dominated outputs from forest sites in the Netherlands and Germany that are subjected to N deposition loadings of 37-60 kg N $ha^{-1}yr^{-1}$. The large quantities of Al were attributed to the high degree of soil acidification in those forest ecosystems.

1.4.3 Nutrient cycling

Nitrogen saturation, because it leads to the excess leaching of nutrient elements such as Mg and Ca, is responsible for serious nutritional imbalances in sensitive ecosystems. Bredemeier *et al.* (1998) reported high Mg losses attributed to high rates of acidification in coniferous sites in the Netherlands. The net loss of Mg observed at these sites presents a serious threat to sustainability of nutrient cycling. At one Dutch site, for example, Mg content in the needles was reported as less than 0.02% while values less than 0.05% are below the minimum Mg nutritional threshold.

1.4.4 Nitrogen form (NH_4 vs. NO_3)

The manner in which excess mineral N behaves in soils depends strongly on the form of N, whether it is oxidised or reduced. Staunes and Kjønaas (1998) reported that increases in NO_3^- leaching resulted from reduced immobilisation rather than enhanced nitrification in a mixed-age coniferous catchment in Sweden. This implies that the input of NO_3^- resulted in immediate leaching and not in the increase of N status of the ecosystem. Gundersen (1998) also found that the form of N deposited in a Denmark site determined the fate of the N.

Although decomposition of organic matter remained unchanged, net N mineralisation was 85% more than the control. Gundersen (1998) concluded that NO_3^- immobilisation had become saturated, and that gross mineralisation had not increased. All of the NH_4^+ inputs were retained in the system whereas the NO_3^- concentration in the soil solution increased.

Emmett *et al.* (1998) also noticed a substantial difference in the fate of excess NO_3^- compared to NH_4^+ that had been added to the system. Comparison between added deposition of NH_4NO_3 and $NaNO_3$, both at loadings of 35 kg N $ha^{-1}yr^{-1}$, revealed lower levels of NO_3^- leaching in the former treatment. The implication is that the input NH_4^+ had been retained in the mineral soil adsorbed on cation exchange sites and/or it was immobilised by the microbial populations. The authors concluded that future increases in oxidised N deposition would result in immediate NO_3^- leaching. The speed with which an ecosystem responds (NO_3^- begins to leach) to NH_4^+ deposition will depend on initial N status of the forest floor and the application rate.

1.5 Conclusions

Nitrogen is an essential nutrient in soil ecosystems the speciation of which is dictated by a variety of microbial processes. The major microbial transformations in soils are nitrogen fixation, mineralisation (ammonification and nitrification), immobilisation and denitrification. The balance between mineral N and organic N in soils is crucial to the stability of the ecosystem. A deficiency in organic N can lead to nutritional imbalances in vegetation and microbial populations, whereas excess organic N can lead to an excess of mineral N, which can have serious ramifications through N leaching and long-term soil and water acidification.

There are a number of factors that will affect the amount of mineral N in soils including temperature, moisture content, aeration, C/N ratio, salinity, mineralogy and texture. In view of the perpetual increases in N deposition in much of Europe and North America, much research has been conducted to determine, qualitatively and quantitatively, the effects of increased N loadings on sensitive ecosystems. A significant relationship between C/N ratio and nitrification rate has been found (Gundersen *et al.*, 1998; Emmett *et al.*, 1998). Evidence has also been presented indicating that deposition can strongly enhance N leaching if NO_3^- immobilisation is saturated, even if NH_4^+ is continually retained in the soil. At the Aber site, Emmett *et al.* (1998) found that, after five years of treatment, all of the NO_3^- that had been

added to the system had been leached. The authors concluded that a change in total N status necessarily pre-empts changes in rates of mineralisation. The 85.7 kg NH_4-N ha^{-1} that had been retained in the system was only sufficient to raise the N content of the forest floor by 0.1%, which was not enough to influence the rates of turnover in the ecosystem. In this way, the oxidation state of the N that is deposited is crucial to sensitive environments. Deposition will, however, only influence the rate of mineralisation inasmuch as it influences the total N pool of the system.

Chapter 2. Soil characterisation with special reference to N status

2.1 Introduction

Recent studies in Southern Africa have documented the impacts of afforestation on soil properties and stream water chemistry draining forest catchments (Morris, 1986; du Toit, 1993; Nowicki, 1997; Sugarman, 1999). Afforestation reportedly induces acidification, reducing acid neutralising capacity and base saturation in sensitive upland soils in South Africa. It has also been noted that afforestation coincides with enhanced NO_3^- concentrations in soil solutions and stream waters draining forest catchments in the eastern escarpment area of South Africa (Nowicki, 1997). This observation, in the light of the current research regarding N saturation in forest ecosystems in the Northern Hemisphere (e.g. Aber *et al.*, 1989 and Gundersen *et al.*, 1998), demands further study of afforestation-induced soil chemical properties.

This chapter presents the results of soil chemical and physical characterisation in terms of pH, exchangeable cations, soil solution chemistry, extractable mineral N and solid phase organic C, total N and texture. The purpose of this chapter is twofold: firstly, to characterise the soils in a general sense and, secondly, to assess differences in soil properties stemming from afforestation with special reference to N status.

2.2 Materials and methods

2.2.1 Study area

Twenty soil samples were taken in August 1999 in the Graskop and Kaapsehoop areas of the eastern escarpment in Mpumalanga province, South Africa. Ten samples were taken at each locality, half of them in grassland and the other half in pine forests. Site details are shown in Table 2.1 and detailed maps showing the position of sampling sites are located in Appendix A.

The study area is characterised by the presence of extensive pine plantations dominated by *Pinus patula*, *P. elliottii* and *P. taeda*. Forests are developed on mountainous terrain with highly leached, naturally acidic soils previously covered by montane grassland (White, 1978).

The eastern escarpment lies within the afro-temperate climatic zone with rainfall predominantly in summer with a mean annual precipitation (MAP) of about 1250-1850 mm (Olbrich and du Toit, 1993). Approximately 200 km from the coast, the eastern escarpment is affected by ocean-derived fogs which impart a maritime signature on the rainfall. Total acid deposition [(SO_4^{2-} + NO_3^-) – total base cations in forest throughfall] is approximately 0.5 $kmol_c ha^{-1} yr^{-1}$, which is in the lower range for polluted areas in North America and Northern Europe (Olbrich and du Toit, 1993).

Sampling sites in the Graskop area lie between 24°51.31' and 24°52.81' S and 30°21.16' and 30°53.56' E and the altitude ranges between 1460 - 1680 m above sea level. The underlying geology consists primarily of fine- to medium-grained quartzite of the Wolkberg group and the undifferentiated upper sediments of the Wolkberg group of the Transvaal Supergroup, as well as medium grained diabase intrusions.

Sampling sites in the Kaapsehoop area lie between 25°34.00' and 25°38.03' S and 30°44.39' and 30°46.59' E and the altitude ranges between 1600 and 1700 m above sea level. The underlying geology is varied with the majority of samples overlying fine- to medium-grained quartzite of the Black Reef Formation of the Transvaal Supergroup. Some samples overlie fine- to medium-grained sandstone, intermediate lava, or arkose conglomerate of the Wolkberg group, compact poorly bedded dolomite of the Malnani group, or hornblende-biotite granite and dolerite/diabase intrusions.

In both sampling regions the complex geology, extensive colluvial redistribution as a result of steep topography and the high degree of weathering in the regolith make it difficult to identify soil parent materials with accuracy.

Table 2.1 Details of soil sampling sites of the Graskop and Kaapsehoop areas of the eastern escarpment, South Africa. na = not applicable.

Sample	*Location*	*Vegetation*	*Age (yrs)**	*Latitude*	*Longitude*	*Aspect*	*Slope type*	*Terrain unit*	*Altitude (m)*	*Geology*	*Soil texture*
ggs1	Graskop	grassland		24°51.92'	30°53.56'	NW	concave	lower slope	1680	quartzite	loamy sand
ggs2	Graskop	grassland		24°51.58'	30°52.85'	na	convex	crest	1620	quartzite	sand
ggs3	Graskop	grassland		24°51.72'	30°52.26'	NW	straight	midslope	1570	quartzite	sand
ggs4	Graskop	grassland		24°51.73'	30°51.94'	W	convex	midslope	1520	quartzite	sand
ggs5	Graskop	grassland		24°52.31'	30°52.00'	NW	convex	upper-midslope	1540	quartzite	sand
gfs1	Graskop	*P. elliottii*	25	24°52.81'	30°52.50'	NW	straight	upperslope	1590	quartzite	loamy sand
gfs2	Graskop	*P. taeda*	30	24°52.05'	30°51.67'	NW	straight	lower-midslope	1520	diabase	loamy sand
gfs3	Graskop	*P. taeda*	30	24°52.57'	30°51.82'	NW	convex	upperslope	1545	quartzite	sand
gfs4	Graskop	*P. taeda*	40	24°52.51'	30°51.47'	W	straight	midslope	1515	quartzite	loamy sand
gfs5	Graskop	*P. taeda*	20	24°52.62'	30°51.16'	NW	straight	midslope	1460	quartzite	loamy sand
kgs1	Kaapsehoop	grassland		25°38.03'	30°44.88'	NW	straight	upper-midslope	1650	quartzite	loamy sand
kgs2	Kaapsehoop	grassland		25°37.54'	30°45.12'	NW	convex	upperslope	1660	granite	loamy sand
kgs3	Kaapsehoop	grassland		25°36.89'	30°44.45'	NW	straight	midslope	1615	quartzite	loamy sand
kgs4	Kaapsehoop	grassland		25°35.57'	30°46.59'	NW	convex	crest	1700	granite	sand
kgs5	Kaapsehoop	grassland		25°35.59'	30°4650'	W	straight	upperslope	1690	diabase	loamy sand
kfs1	Kaapsehoop	*P. patula*	10	25°37.97'	30°44.73'	NW	straight	upper-midslope	1640	quartzite	loamy sand
kfs2	Kaapsehoop	*P. patula*	10	25°37.43'	30°45.03'	NW	convex	upperslope	1660	diabase	sandy loam
kfs3	Kaapsehoop	*P. patula*	40	25°37.76'	30°44.39'	N/NW	convex	crest	1650	dolomite	sandy loam
kfs4	Kaapsehoop	*P. patula*	20	25°36.88'	30°44.41'	NW	straight	midslope	1600	quartzite	sandy loam
kfs5	Kaapsehoop	*P. patula*	20	25°34.00'	30°45.27'	NW	straight	midslope	1630	conglomerate	sandy loam

* Approximate age of pine stands

2.2.2 Sample collection and preparation

Samples were taken as composites of about five individual samples in each field from the top 20-25 cm of soil. Previous studies in the area have purposefully excluded the fresh litter, partially decomposed litter layer and subjacent organic layers of forest samples (Nowicki, 1997 and Sugarman 1999). Due to the well-documented role of the forest floor in nutrient cycling in forest ecosystems (e.g. McColl and Gressel, 1995) the current study included the partially decomposed litter layer and subjacent organic layers in the forest soil sampling. The forest samples, therefore, consisted of combined organic layers and upper mineral soil to a depth of approximately 20-25 cm, whereas the grassland samples consisted of the upper mineral soil (an organic litter layer was for the most part absent).

Approximately three-quarters of each sample was air-dried, crushed and the < 2 mm fraction was separated and stored for analysis. The remaining quarter of each sample was passed through a 2-mm sieve and refrigerated at about 4°C in order to maintain field-moist conditions and to inhibit microbial transformations. Refrigerated samples were used for 2 M-KCl extractable NO_3^- and NH_4^+ analyses and N mineralisation experiments (Chapter 3). The remaining analyses were performed on the air-dried samples. The moisture content of the air-dried and field-moist samples was determined gravimetrically by heating at 110°C for at least 12 hours. The moisture content was subsequently used as a correction factor accordingly.

2.2.3 Analytical techniques

Analyses were done at the Department of Geological Sciences, University of Cape Town with the following exceptions: oxidisable C, NH_4OAc extractable base cations and texture determination were done at Infruitech and total N was done at the University of Stellenbosch. Organic carbon content and total N were determined with the Walkley-Black wet oxidation technique (Nelson and Sommers, 1982) and the Kjeldahl digestion method (Bremner and Mulvaney, 1982), respectively. Particle size was determined by the hydrometer method according to Gee and Bauder (1986). Extractable base cations (Na^+, K^+, Mg^{2+} and Ca^{2+}) were determined by atomic absorption spectroscopy in 1 M NH_4OAc extracts according to Thomas (1982). Extractable acidity was determined by potentiometric titration of 1 M KCl extracts with NaOH to the phenolphthalein end point (pH 8.3) (Rowell, 1994). Extractable NH_4^+ and NO_3^- were determined in 2 M KCl extracts according to the indo-phenol blue (Keeney and

Nelson, 1982) and copperized cadmium reduction (Stock, 1983) colorimetric methods, respectively. Soil pH was measured in 1:2.5 suspension of both deionized H_2O and 1 M KCl. Saturated paste extracts were obtained according to Rhoades (1982) and the extracts were analysed for EC, pH, major cations and anions by ion chromatography, and trace elements by inductively coupled plasma mass spectrometry (ICP-MS). Further details regarding analytical methods and an appraisal of the rigor of the techniques and quality of the data are presented in Appendix B.

2.2.4 Statistical interpretation techniques

All statistical calculations and graphics were carried out using the STATISTICA computer programme version 5.1 (StatSoft Inc., 1998).

Non-parametric statistics were chosen for interpretation because the data, for the majority of variables, do not conform to a normal distribution and because of the relatively small sample sizes being evaluated.

Correlation between variables was assessed with the Spearman's correlation coefficient (r_s values) which consists of ranking raw data values independently for each variable comprising a data set for the n cases consisting of n pairs of ranks. The squared differences in the ranks is summed and used to calculate r_s. Spearman's correlation coefficient is the non-parametric equivalent of Pearson's r and is approximately 90% as efficient.

Cluster analysis was performed on all samples and on sub-set samples from both localities in order to determine the legitimacy of imposing vegetation as a clustering variable (section 2.4.2). Hierarchical tree cluster analysis consists of an algorithm that computes the geometric distance in multi-dimensional space (Euclidean distance) between cases based on raw data. Subsequently, cases are joined into successively larger clusters based on Euclidean distances.

In order to assess for which variables there is a significant difference between the two vegetation types the Mann-Whitney U test was employed. This is the non-parametric equivalent of the t-test and is approximately 95% as efficient. The statistic consists of assigning ranks to median values for all cases, summing the ranks of each group and comparing the summed ranks between groups. Statistical significance is assessed by p-values which indicate the probability of error in accepting the test result. Mann-Whitney U results

are presented graphically with box and whisker plots which show the median, inter-quartile range (box), non-outlier maximum and minimum values (whiskers), outliers and extremes. Outlier values are defined as follows:

$$> [75^{th} \text{ percentile} + 1.5(\text{inter-quartile range})], \text{ or}$$

$$< [25^{th} \text{ percentile} - 1.5(\text{inter-quartile range})].$$

Extreme values are defined as:

$$> [75^{th} \text{ percentile} + 2(\text{inter-quartile range})], \text{ or}$$

$$< [25^{th} \text{ percentile} - 2(\text{inter-quartile range})].$$

2.3 Results and discussion

A complete data set summarising the results of the soil characterisation is presented in Table 2.2.

2.3.1 Soil chemical assessment

2.3.1.1 Soil solution composition

Saturated paste extracts were used as a basis for estimating the composition of the soil solution. The ionic composition of the extracts is presented in Figures 2.1 and 2.2 (Graskop samples) and Figures 2.3 and 2.4 (Kaapsehoop samples). The cations are generally dominated by Na^+ and K^+, followed by lesser amounts of Ca^{2+}, Mg^{2+} and NH_4^+ (generally in that order). The anion composition is dominated by Cl^-. The dominance of Na^+ and Cl^- is expected due to a maritime influence. The dominance of K^+ is unusual: due to its ideal size to charge ratio K^+ is often selectively associated with vermiculite and micaceous minerals on cation exchange sites and, therefore, often partitioned to the solid phase of soil (McBride, 1994). In these highly leached, clay poor, quartzitic samples it is possible that the lack of suitable exchange sites accounts for the apparently anomalous high K^+ concentrations. It is interesting to note that there is a highly significant correlation between K^+ and Cl^- in all samples ($r_s = 0.77$, $p < 0.001$) exceeding the usual strong correlation between Na^+ and Cl^- ($r_s = 0.43$, $p = 0.06$).

Table 2.2 Characterisation of grassland and forest soil samples from Graskop and Kaapsehoop areas of the eastern escarpment area of South Africa

Location	Graskop										Kaapsehoop									
Vegetation	Grassland					Forest					Grassland					Forest				
Sample ID	ggs1	ggs2	ggs3	ggs4	ggs5	gfs1	gfs2	gfs3	gfs4	gfs5	kgs1	kgs2	kgs3	kgs4	kgs5	kfs1	kfs2	kfs3	kfs4	kfs5
pH (H_2O)	4.72	4.86	4.76	5.05	5.23	4.58	4.69	4.28	4.76	4.47	5.28	5.24	5.02	4.55	4.70	4.89	4.68	3.26	4.49	4.93
pH (KCl)	4.19	3.85	4.09	4.18	4.20	3.97	3.94	3.61	4.05	3.76	4.29	4.28	4.31	3.73	4.14	4.20	4.07	2.72	3.83	4.01
NH_4OAc extractable cations ($mmol_c kg^{-1}$)																				
K	1.80	0.31	0.76	0.30	0.62	1.05	0.55	0.51	0.89	0.31	1.22	1.56	1.11	0.30	1.15	0.72	0.94	1.14	0.94	0.93
Ca	3.85	1.23	2.37	0.70	1.44	1.15	2.20	1.23	5.59	1.02	4.98	5.81	4.09	1.30	1.88	3.42	2.20	2.01	5.72	2.07
Mg	2.28	0.21	0.96	0.10	0.31	7.34	0.55	0.26	2.01	0.10	1.02	2.91	1.27	0.00	0.42	1.14	1.78	3.81	2.08	10.7
Na	1.20	0.31	0.35	0.20	0.51	0.94	0.55	0.61	1.23	0.51	0.61	1.14	1.00	0.20	0.31	0.72	0.73	1.91	0.94	1.03
1 M KCl extractable acidity ($mmol_c kg^{-1}$)																				
Acidity	30.9	6.5	17.8	5.2	14.8	50.4	52.8	41.9	50.5	28.7	8.1	16.5	20.7	9.1	21.2	20.9	33.5	123.1	43.8	30.4
CEC_e[1]	40	9	22	7	18	61	57	45	60	31	16	28	28	11	25	27	39	132	53	45
Acid saturation (%)[2]	77	76	80	80	84	83	93	94	84	94	51	59	74	83	85	78	86	93	82	67

[1] CEC_e (effective cation exchange capacity) = Σ NH_4OAc base cations + KCl acidity

[2] Acid saturation % = KCl extractable acidity ÷ (Σ NH_4OAc base cations + KCl acidity) x 100

Table 2.2 Soil characterisation (continued)

Sample ID	ggs1	ggs2	ggs3	ggs4	ggs5	gfs1	gfs2	gfs3	gfs4	gfs5	kgs1	kgs2	kgs3	kgs4	kgs5	kfs1	kfs2	kfs3	kfs4	kfs5
Saturated paste extract																				
EC (µS/cm)	227	288	313	267	344	205	184	187	224	196	245	189	256	220	146	201	243	298	231	281
pH	6.94	6.05	6.24	6.72	6.65	5.22	6.23	4.83	6.09	5.72	6.07	5.98	6.21	6.02	6.16	6.01	6.09	3.52	5.16	5.40
Ions ($mmol_c l^{-1}$)																				
Na	0.17	0.61	0.83	0.69	0.68	0.66	0.49	0.58	0.56	0.69	0.65	0.53	0.73	0.41	0.38	0.36	0.54	0.70	0.57	0.81
NH_4	0.076	0.38	0.17	0.15	0.10	0.093	0.049	0.091	0.15	0.074	0.24	0.10	0.25	0.13	0.062	0.14	0.20	0.12	0.16	0.063
K	2.59	0.64	0.83	0.87	1.38	0.50	0.41	0.30	0.32	0.47	0.71	0.34	0.50	0.48	0.30	0.42	0.63	0.21	0.31	0.34
Mg	0.27	0.094	0.31	0.23	0.11	0.14	0.15	0.18	0.23	0.14	0.24	0.23	0.19	0.26	0.13	0.16	0.25	0.41	0.25	0.90
Ca	0.52	0.28	0.68	0.46	0.33	0.33	0.30	0.28	0.34	0.33	0.37	0.32	0.30	0.35	0.23	0.00	0.33	0.34	0.34	0.36
Al ($mmol\ l^{-1}$)	0.005	0.011	0.019	0.020	0.023	0.017	0.001	0.063	0.010	0.050	0.024	0.004	0.002	0.011	0.007	0.007	0.009	0.27	0.036	0.033
F	0.053	0.045	0.040	0.029	0.047	0.028	0.039	0.021	0.031	0.019	0.062	0.018	0.042	0.042	0.022	0.030	0.056	0.057	0.057	0.040
Cl	1.19	1.36	1.46	1.19	2.18	1.20	0.92	0.84	1.17	1.12	1.22	0.83	1.05	0.75	0.54	0.83	1.25	0.76	1.00	1.18
NO_3	0.036	0.055	0.055	0.056	0.070	0.075	0.15	0.061	0.21	0.041	0.057	0.037	0.56	0.35	0.19	0.19	0.055	bdl[3]	0.077	0.064
SO_4	0.19	0.59	0.39	0.44	0.24	0.17	0.14	0.23	0.15	0.16	0.39	0.24	0.14	0.36	0.18	0.18	0.46	0.63	0.54	0.72

[3] bdl - below detection limit, reported as 0.1 mg/l for NO_3^- by HPIC (Standard Methods, 1995).

Table 2.2 Soil characterisation (continued)

Sample ID	ggs1	ggs2	ggs3	ggs4	ggs5	gfs1	gfs2	gfs3	gfs4	gfs5	kgs1	kgs2	kgs3	kgs4	kgs5	kfs1	kfs2	kfs3	kfs4	kfs5
2 M KCl extractable mineral N ($mmol_c kg^{-1}$)																				
NO_3	0.19	0.17	0.15	0.15	0.14	0.27	0.28	0.25	0.41	0.14	0.08	0.14	0.72	0.41	0.54	0.53	0.18	0.16	0.34	0.14
NH_4	0.32	0.13	0.25	0.27	0.41	0.45	0.75	0.40	0.58	0.35	0.19	0.27	0.69	0.73	0.75	0.58	0.21	0.00	0.32	0.50
Solid phase																				
Total N (%)	0.40	0.12	0.15	0.05	0.17	0.45	0.33	0.22	0.48	0.14	0.17	0.29	0.58	0.08	0.41	0.21	0.35	0.23	0.16	0.15
Organic C (%)	10.6	2.8	2.8	1.0	3.5	9.2	8.6	4.5	9.8	3.4	3.3	5.3	9.7	1.4	6.6	3.9	6.3	8.2	6.6	4.4
C/N[4]	26	24	19	22	21	20	26	20	20	24	19	18	17	18	16	19	18	36	41	29
Texture (%)																				
Clay	4.0	1.8	2.6	1.1	2.4	4.4	4.2	2.0	2.6	4.2	3.8	4.8	4.2	1.3	6.2	4.4	8.2	15.6	7.2	17.3
Silt	11.2	4.8	6.8	3.0	8.2	12.2	14.8	7.0	15.6	10.4	11.0	17.8	19.6	4.7	15.2	11.4	14.6	28.8	20.8	22.3
Fine sand	75.2	29.6	26.0	17.3	31.4	58.2	64.0	36.8	59.4	26.2	44.2	58.0	59.6	12.8	50.6	37.6	59.4	48.8	64.6	37.8
Medium sand	3.2	35.8	42.2	44.4	39.0	16.0	10.2	36.8	8.8	35.2	33.8	6.8	9.2	58.3	9.6	29.6	8.8	2.0	4.4	8.4
Coarse sand	6.4	28.0	22.4	34.2	20.0	9.2	6.9	17.4	13.6	24.0	7.2	12.6	7.4	22.9	18.4	17.0	9.0	4.8	3.0	14.2

[4] C/N = organic carbon % ÷ total nitrogen %

The anion suite is dominated by Cl^- followed by accessory concentrations of SO_4^{2-}, NO_3^- and F^-, generally in that order. As previously stated, the dominance of Cl^- may be attributed to a maritime influence. Alkalinity is not expected to contribute to the anionic composition of the soil solution due to the negligible dissociation of H_2CO_3 as pH approaches 5 (Ulrich, 1991). The suite of anions may not, however, be complete because dissolved organic matter (DOM), which often contributes to the anionic composition of a soil solution (particularly in soils rich in organic carbon such as these) has not been analysed. This could contribute to the apparent charge imbalance in the soil solutions; this is discussed further in Appendix B. DOM may be expected to contribute significantly to the speciation and ultimately the availability of environmentally important constituents, particularly Al^{3+} and Mn^{2+}, in these extremely acidic soils (Ulrich, 1991; Sumner *et al.*, 1991).

It is important to emphasise the consistently low ionic strength of these solutions. The EC of the samples ranges from 145 - 344 μScm^{-1} with a median of 229 μScm^{-1}, which is generally low for soil solutions indicative of the highly leached status of the soils (Donkin and Fey, 1993). This may also contribute to the charge imbalance as small errors in analysis constitute a large percentage of the total ionic strength.

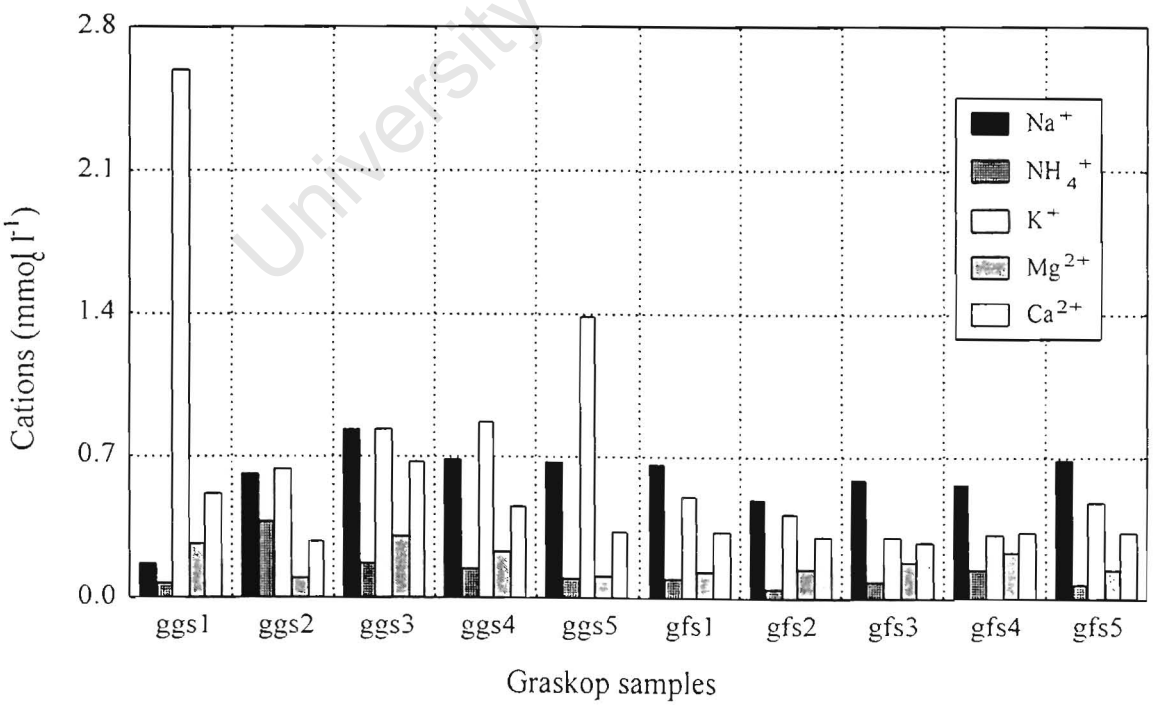


Figure 2.1 Cation composition of saturated paste extracts for all Graskop samples

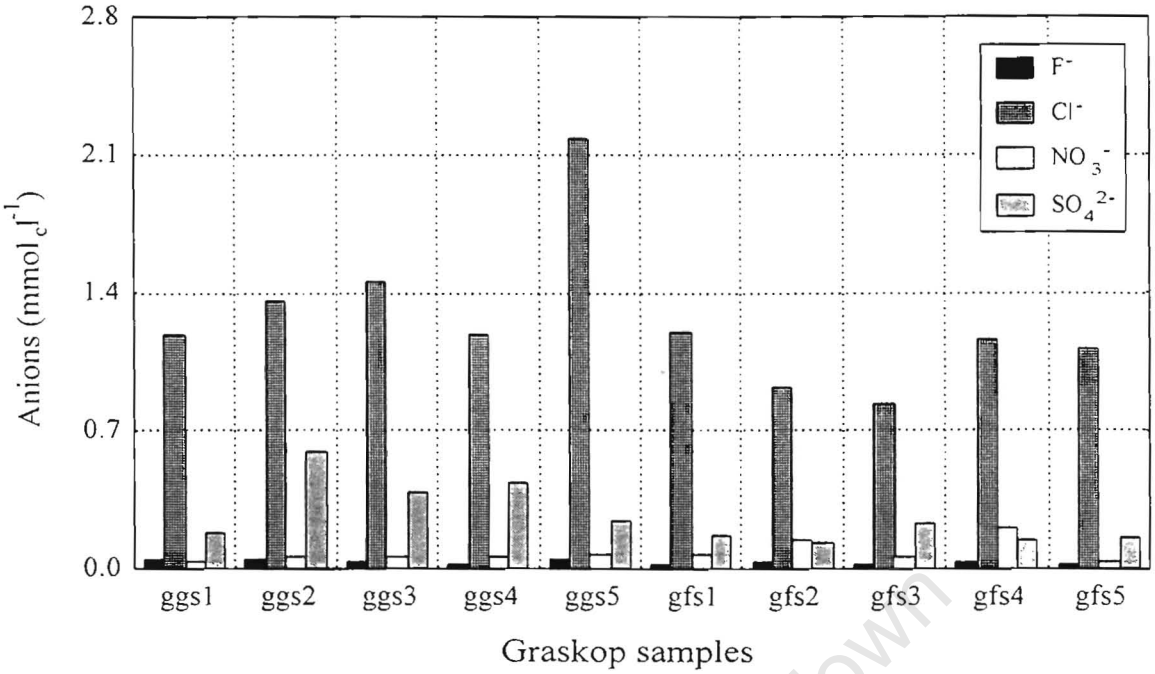


Figure 2.2 Anion composition of the saturated paste extracts for all Graskop samples

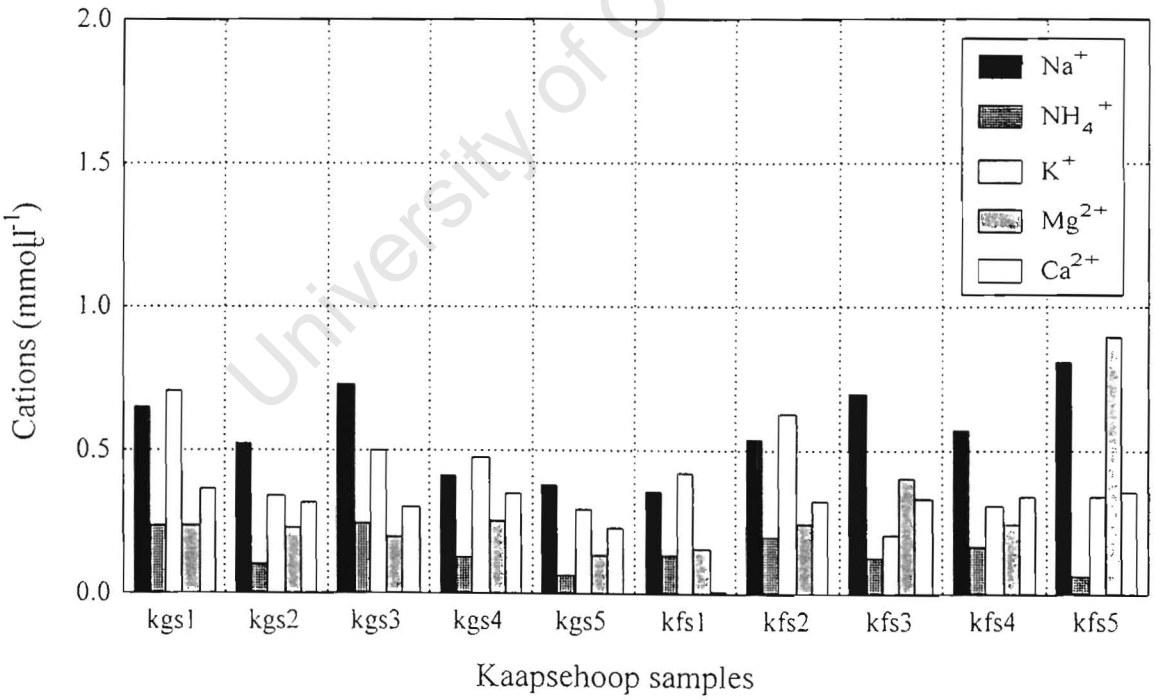


Figure 2.3 Cation composition of saturated paste extracts for all Kaapsehoop samples

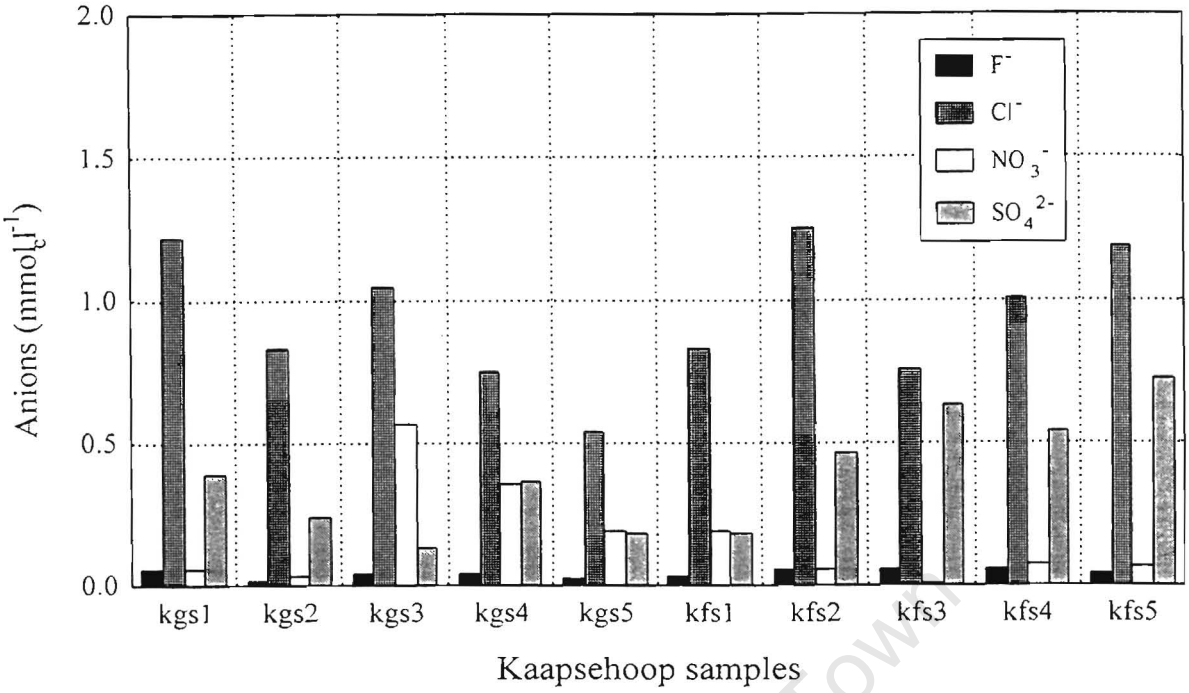


Figure 2.4 Anion composition of saturated paste extracts for all Kaapsehoop samples

2.3.1.2 Acidity status

Acidity has been termed a major limitation to soil productivity in much of the world (McBride, 1994), despite being a natural consequence of weathering reactions and leaching, atmospheric and biological CO_2 inputs, and phytocycling of nutrients by vegetation in many soil environments (McBride, 1994; Sumner *et al.*, 1991). Agricultural practices, specifically afforestation, however, intensify natural acidification processes around the world in general (Knoepp and Swank, 1994) and topically in Southern Africa (Nowicki, 1997; Morris 1986; Sugarman 1999). It is, therefore, necessary to consider acidity critically, particularly when evaluating impacts of afforestation on soil chemical properties (section 2.3.3.1). Acidity has been evaluated in terms of active acidity (soil pH) and extractable acidity (extractable with a concentrated neutral salt), both of which are important characteristics of a soil.

pH

The pH of the samples was measured in deionized H_2O, 1 M KCl and in saturated paste extracts (SPE). It was necessary to measure the pH in a concentrated solution of a neutral unbuffered salt (KCl) to compensate for the dilution effect. According to Alloway (1995)

measuring the pH of the supernatant of a soil/H_2O mixture typically results in values 1 to 1.5 units higher than that of the soil solution near the solid surfaces. This is because H^+ ions replace other cations on exchange surfaces thus depleting the activity of H^+ in the supernatant and concentrating the protons in a diffuse layer near the soil surface. It is expected that a sample with a higher cation exchange capacity (CEC) would have a greater ΔpH [pH (H_2O) – pH (KCl)] because there are more exchange sites on which the H^+ ions can replace other cations, therefore demonstrating a larger dilution effect.

All of the samples are characterised by low pH. Table 2.2 lists all three pH values for all samples. The range of pH (KCl) is 2.72-4.31 with a median of 4.06; pH (H_2O) is 3.26-5.28 with a median of 4.74; the range for pH (SPE) is 3.52-6.94 with a median of 6.09. The extremely low pH values are attributed to the highly leached status of the soils and are expected to be compounded by the prevalence of poorly buffered quartzitic parent material (Ulrich, 1991). The elevated values of pH taken in saturated paste extracts relative to that taken in H_2O and KCl may be due, in part, to the degassing of CO_2 which results from the suction extraction. Removal of CO_2 from the system results in a loss of H_2CO_3, which is borne out in an increase in pH. Due to the uncertainties associated with possible degassing of CO_2 and an equilibrium delay following the separation of solution and solid, further consideration of pH (SPE) will be discarded and discussion will focus solely on pH (H_2O) and pH (KCl). It may have been more instructive to measure the pH in the saturated paste itself, rather than in the extract (Rowell, 1994).

The relationship between pH (KCl) and pH (H_2O) is presented in Figure 2.5. As expected, there is significant positive correlation between the variables (r_s = 0.85, $p < 0.001$) and the line of best-fit has a slope of 0.76. Inspection of the relationship reveals an apparent decrease in ΔpH as pH decreases; the implication is that as pH decreases the relative CEC also decreases, minimising the dilution effect. In fact, solving the reference equation ($x = y$) and the trendline ($y = 0.76x + 0.37$) concurrently reveals that at pH 1.54, ΔpH = 0 indicating no dilution effect. This is the theoretical pH at which positive exchange sites would equal negative exchange sites, i.e. the point of zero charge (Sposito, 1989). Below pH 1.54 ΔpH would become negative indicating a net positive charge on the soil surface. The apparent decrease in ΔpH with decreasing pH can be attributed to the influence of variably charged ion exchange surfaces, namely oxyhydroxides and organic matter. As pH decreases variably charged surfaces become protonated (or complexed with Al^{3+}) resulting in fewer negatively charged and more positively charged anion exchange surfaces. A similar trend was described

by Fey *et al.* (1998) who suggested that acidification resulting from pine and tea plantations may result in an alteration in surface charge with CEC and anion exchange capacity (AEC) becoming more equal in magnitude. Alternatively, the authors proposed that the decrease in ΔpH may just be an artefact associated with normalising ionic strength with 1 M KCl, as the ionic strength in aqueous suspension is comparatively variable and may have a significant effect on pH (H_2O). The possibility that these topsoil samples exhibit (AEC) is examined in Chapter 3. Despite this apparent trend of decreasing ΔpH at lower pH, it is important to recognise the limited range in data points. With the exception of one sample (kfs3), the range of pH (H_2O) is 4.28-5.28 (1.0 pH unit) and the range of pH (KCl) is 3.61-4.31 (0.7 pH unit).

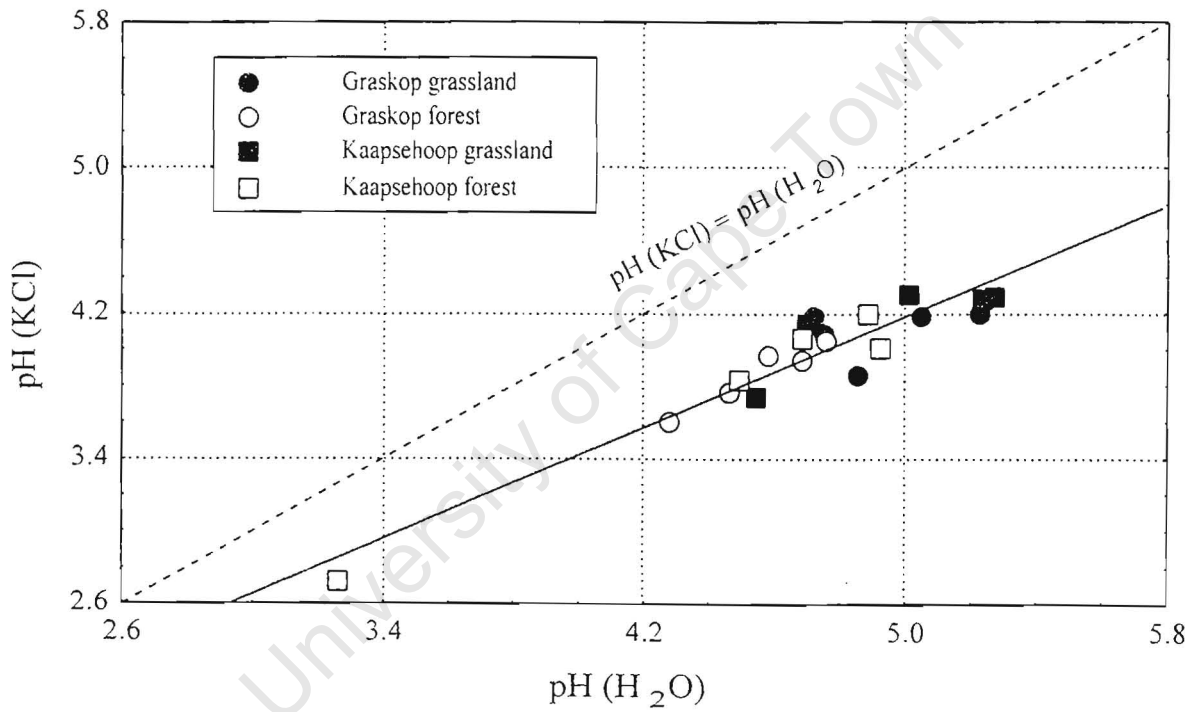


Figure 2.5 Relationship between pH (KCl) and pH (H_2O) for all samples, best-fit line: pH (KCl) = 0.76pH (H_2O) + 0.37.

In general all soils are acidic to strongly acidic in terms of their measured pH values. McBride (1994) reports that soil pH values below 5.0 - 5.5 may be indicative of soluble levels of Al^{3+} and Mn^{2+} that can be biologically toxic. Runge and Rode (1991) indicate that acid soils, such as these, are expected to be characterised by low activity of Ca ions, high activities of H, Al and Mn ions and high NH_4/NO_3 ratios.

Extractable cations

Extractable base cations were determined in NH_4OAc extracts. The results for the Graskop samples are presented in Figure 2.6 and the Kaapsehoop samples in Figure 2.7. The dominant extractable base cation is generally Ca^{2+} and occasionally Mg^{2+}, followed by approximately equal concentrations of K^+ and Na^+. Comparison of extractable base cations and cationic composition of the soil solution reveals an interesting juxtaposition with monovalent dominance (Na^+ and K^+) of the soil solution and divalent (Ca^{2+} and Mg^{2+}) dominance on exchange surfaces. This phenomenon illustrates the concentration-charge rule, which explains the thermodynamic tendency for higher-valent cation adsorption in preference to lower-valent adsorption at lower electrolyte concentrations. The preference for higher-valent cations at low electrolyte concentration arises from a favourable increase in entropy of the exchange system and not energy (enthalpy) considerations (McBride, 1994).

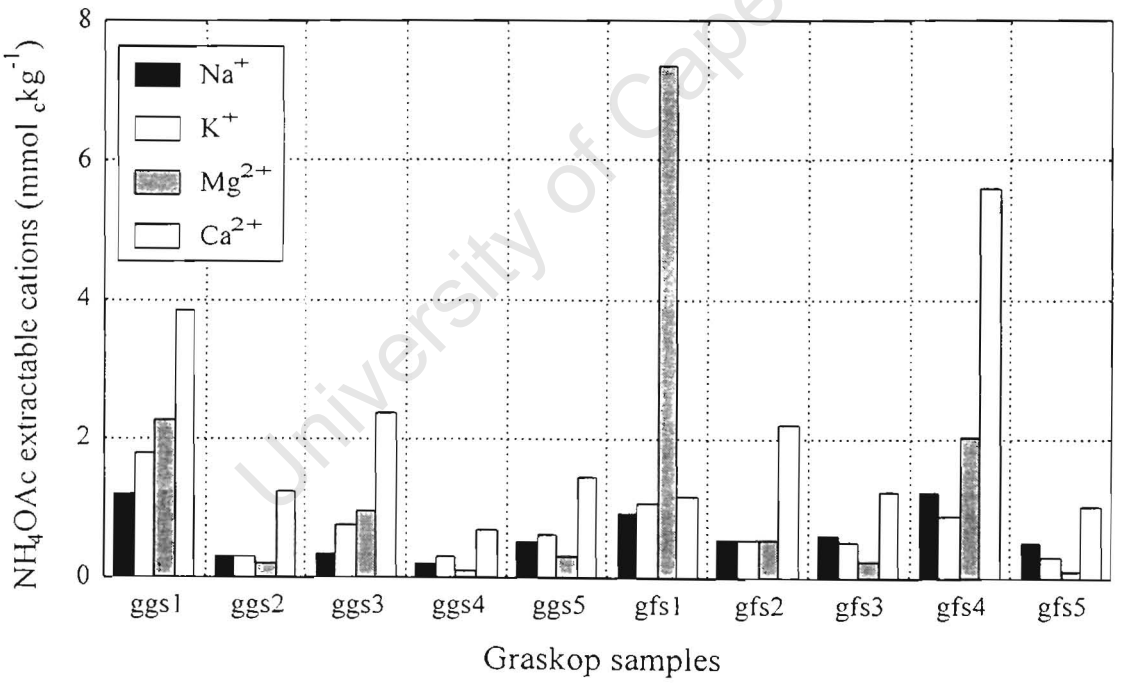


Figure 2.6 NH_4OAc extractable base cations (Na^+, K^+, Mg^{2+} and Ca^{2+}) for Graskop samples

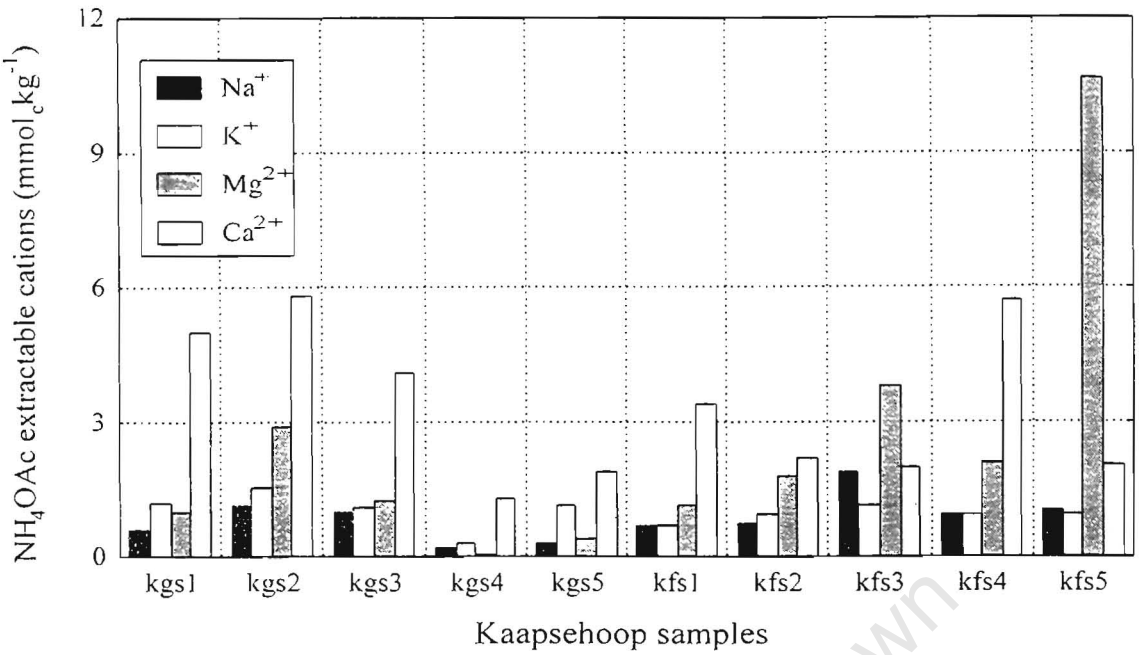


Figure 2.7 NH_4OAc extractable base cations (Na^+, K^+, Mg^{2+} and Ca^{2+}) for Kaapsehoop samples

In general, there are limited amounts of base cations on exchange surfaces compared to acidic cations (discussed below) with a range of total extractable base cations for all samples of 1.3 - 14.7 and a median value of 5.8 $mmol_c kg^{-1}$. The Graskop samples generally have lower total extractable base cations with a range of 1.3 - 10.5 and a median of 3.4 $mmol_c kg^{-1}$ compared to Kaapsehoop samples that range from 1.8 - 14.7 with a median of 7.7 $mmol_c kg^{-1}$.

Extractable acidity (an aggregate measure of exchangeable acidic cations) was measured potentiometrically in 1 M KCl extracts. The results for all the samples are presented in Table 2.2. In general, the samples are characterised by a large amount of extractable acidity with a range of 5.2 - 123 and a median of 25.0 $mmol_c kg^{-1}$ which translates to greater than four times more extractable acidity than total extractable base cations. Nowicki (1997) reported similar values for KCl extractable acidity for 13 pairs of grassland and forest sites in the eastern escarpment of Mpumalanga ranging from 6 - 46 $mmol_c kg^{-1}$.

Derived parameters: CEC_e, acid saturation

Effective cation exchange capacity was calculated as the sum of the extractable base cations plus extractable acidity. The CEC_e of these samples is low (range of CEC_e is 6.5 - 131.9 with a median value of 29.4 $mmol_c kg^{-1}$) reflecting the highly weathered status and low clay

content of the soils (Sposito, 1989). The range of clay content (Table 2.2) is 1.1 - 17.3% with a median of 4.2%. The range of organic carbon content for all the samples is 1.0 - 10.4% with a median of 4.9%. Comparing the correlations between CEC_e and clay content (Figure 2.8) and CEC_e and organic carbon content (Figure 2.9) reveals a more significant relationship between organic carbon content ($r_s = 0.77$, $p < 0.001$), as opposed to clay content ($r_s = 0.59$, $p = 0.006$), with CEC_e. This suggests that the cation exchange capacity of the samples results largely from negatively charged surfaces associated with organic matter. Further inspection of Figure 2.9 reveals an interesting separation between vegetation types illustrated by the two variables; this will be discussed in section 2.3.3.1.

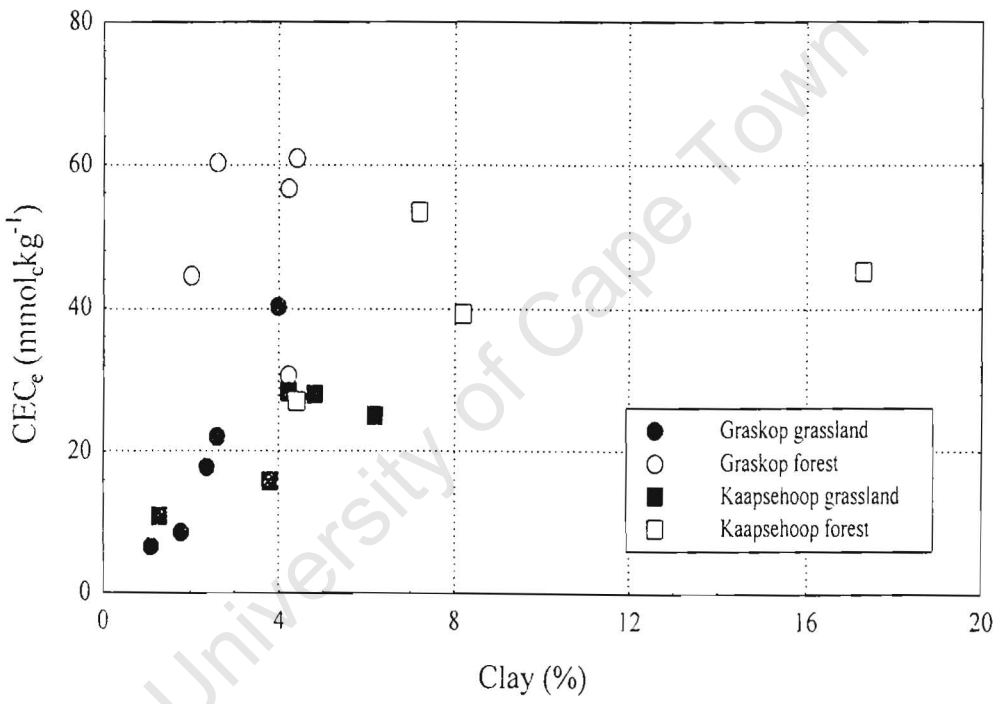


Figure 2.8 Relationship between CEC_e and percentage clay content, $r_s = 0.59$, $p = 0.006$ (sample kfs3 is off scale: x,y co-ordinates: 15.6, 131.9)

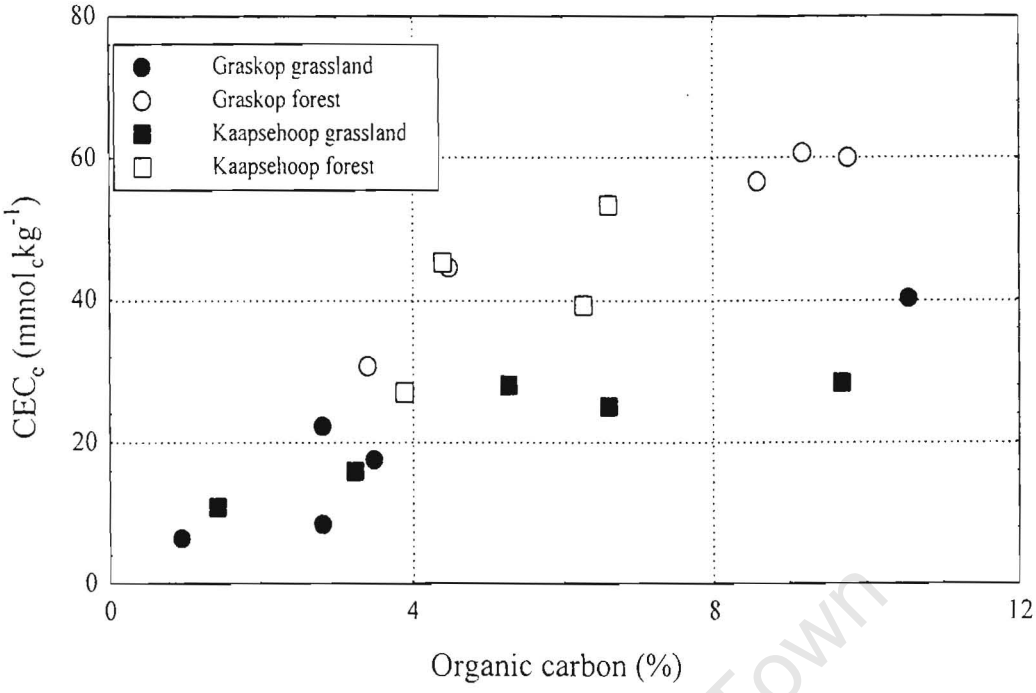


Figure 2.9 Relationship between CEC_e and percent organic carbon content, r_s = 0.77, $p < 0.001$ (sample kfs3 is off scale: x,y co-ordinates are 8.23, 131.9)

An index of acid saturation - relative amount of exchange sites occupied by acidic cations - was calculated from the extractable base cation and acidity data. The trend between acid saturation and pH, shown in Figure 2.10, illustrates a general increase in acid saturation with decreasing pH, as expected. Acid saturation values range between 50-94 % with a median value of 82%, values indicative of severely acidic conditions. Acid saturation of 85% has been reported as a critical threshold with respect to acid stress for acid-tolerant vegetation, above which seasonal nitrification/acidification pulses can lead to strong increases in Al concentration in the soil solution (Ulrich, 1991).

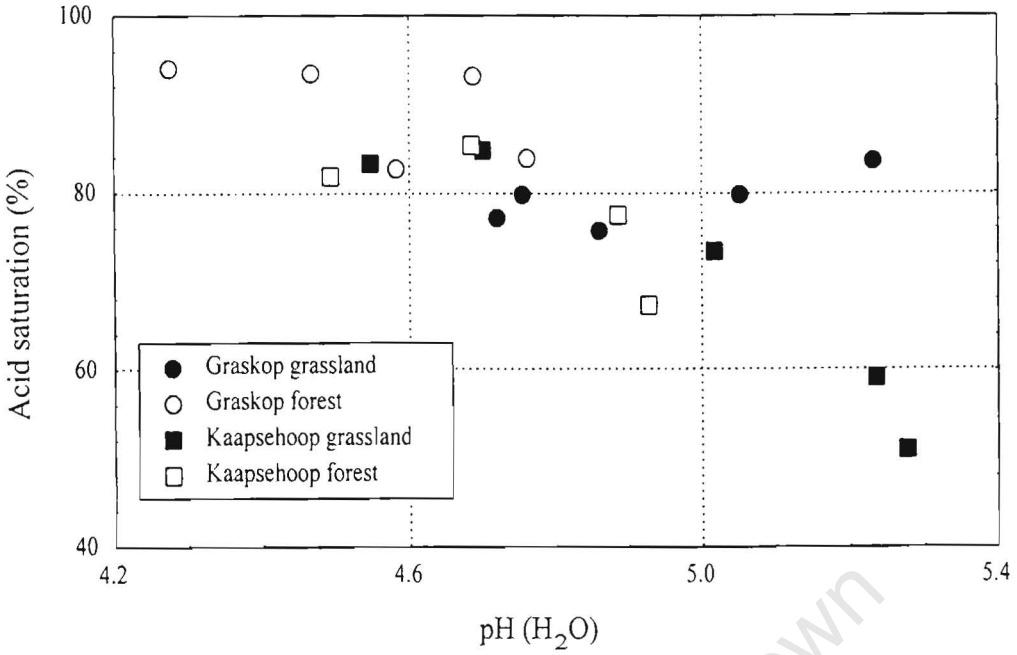


Figure 2.10 Relationship between acid saturation and pH (H_2O) (sample kfs3 is off scale: x,y co-ordinates are 3.26, 93.3)

2.3.1.3 Aluminium

Aluminium is an environmentally important constituent because of its virtually universal role in soil minerals and its tendency to become soluble at low (and high) pH leading to mobility which can result in biological toxicity. Since the soils in this study are extremely acidic, Al was considered important in the general chemical assessment of the soil solution. Aluminium was determined quantitatively by ICP-MS. Since ICP-MS determines total Al, and not exclusively monomeric Al, the likelihood of colloidal contamination needs to be considered carefully. This is particularly likely for samples with pH>5 because of Al hydrolysis and subsequent polymerisation (Bruggenwert *et al.*, 1991).

The speciation of Al as well as the activity of Al^{3+} was calculated using PHREEQC modelling programme (Parkhurst, 1995) with the WATEQ4F database (Ball and Nordstrom, 1995). The pAl of the soil solution was plotted against pH in Figure 2.11. The pAl values correspond to relatively low activities of Al^{3+} indicating undersaturation with respect to gibbsite for the data points falling above the solubility line. Figure 2.11 and subsequent discussion regarding Al solubility, speciation and saturation indices only serve as an indication of the complex Al chemistry likely governing soil properties. Particularly, the omission of DOC from the analyses needs consideration since DOM is known to form strong complexes with Al and

hence to increase total monomeric Al concentration in the soil solution (Paterson *et al.*, 1991). DOM is reported as having an interesting dual effect on Al in soils: it has a general solubilizing effect on Al augmenting mobility, yet simultaneously masking Al toxicity (Ulrich, 1991).

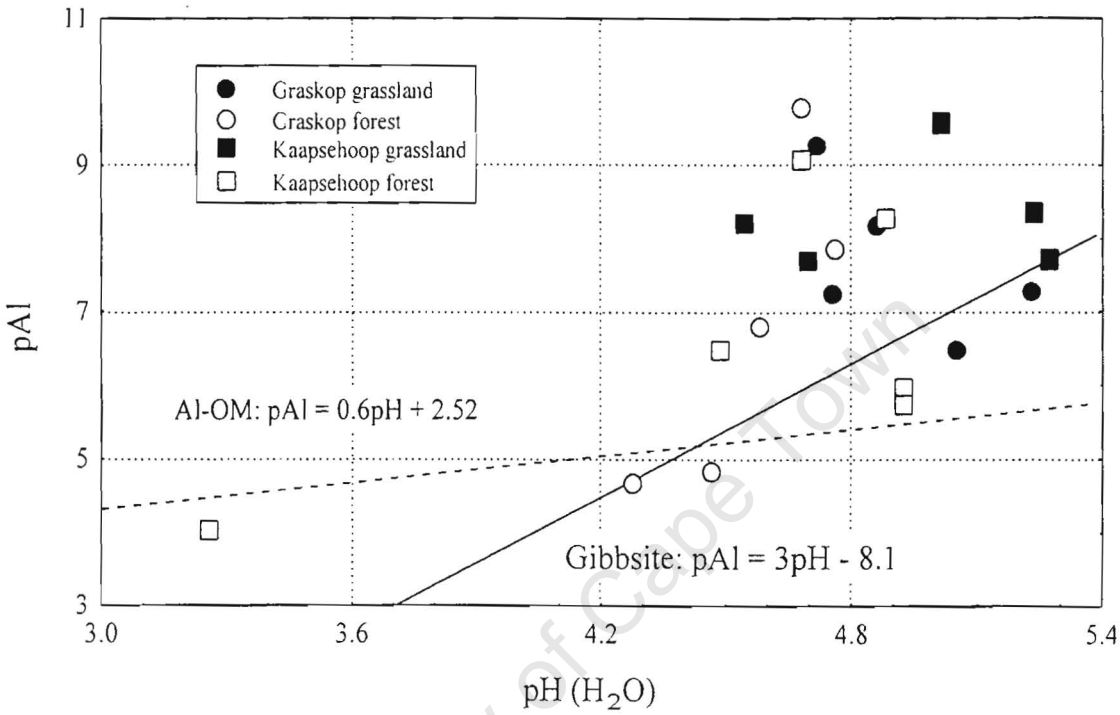


Figure 2.11 Plot illustrating the relationship between pAl and pH (H_2O).

There is evidence that in strongly acidified forest soils (pH < 4.5) solid phase Al complexed organic matter (Al-OM) may control Al solubility and Al^{3+} activity in solution (Cronan *et al.*, 1986; Mulder and Stein, 1994; Fey *et al.* 1998). Fey *et al.* (1998) proposed in their study of humic, ferallitic topsoils taken from paired sites, representing the effects of afforestation or tea production, that the buffer capacity of the acidic soils is linked to organically bound Fe and Al. The authors concluded that the buffering capacity of the highly weathered soils is more closely linked to the presence of organic - sesquioxide complexes than it is to the total content of organic matter, sesquioxides or clay as might be expected.

Figure 2.12 shows exchangeable Al (extractable acidity) plotted against organic carbon. Extractable acidity, as previously mentioned, is an indication of aggregate exchangeable acidic cations. It is the H^+ ions present in the extract resulting from hydrolysis of acidic constituents. The majority of acidic cations associated with exchange surfaces in soils are

various hydrolysed forms of Al (e.g. Al^{3+}, $Al(OH)^{2+}$, $Al(OH)_2^{+}$). Except in very acid soils, exchangeable H^+ is found only in small amounts because clay minerals react with exchangeable H^+ to produce exchangeable Al^{3+} (Rowell, 1994). Gubevu (1997), for example, reported a good correlation between 0.1 M $BaCl_2$-extractable Al and 1 M KCl-extractable acidity ($r_s = 0.97$, $p < 0.001$). In order to assess the role of organic matter in influencing Al solubility, 1 M KCl extractable acidity was used as an index of exchangeable Al for interpretative purposes. The positive correlation between exchangeable Al and organic matter is significant ($r_s = 0.75$, $p < 0.001$). Furthermore, it is interesting to note that there are two distinct trends for grassland samples and forest samples. The slope of the grassland sample trend is 2.1 and the correlation between organic carbon and exchangeable Al is $r_s = 0.81$, $p < 0.001$, whereas the slope of the forest trend is 6.7 and the correlation between the two variables is $r_s = 0.87$, $p < 0.001$. These data suggest that there is a difference in organic matter quality between the two vegetation types, with forest soil organic matter containing substantially more exchangeable Al than grassland soil organic matter. This is explored in more detail in section 2.3.3.1.

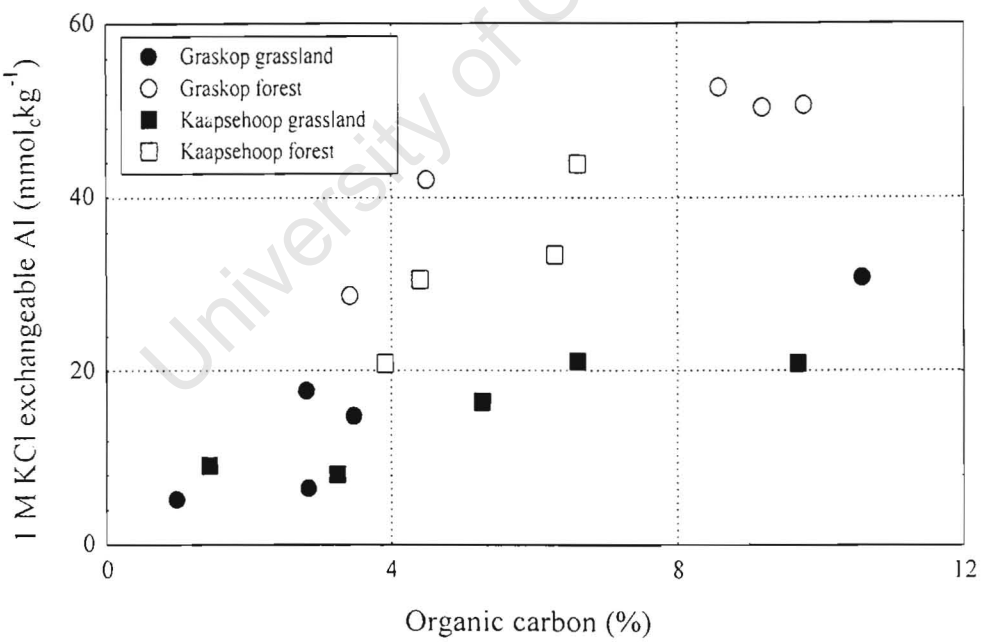


Figure 2.12 Relationships between exchangeable Al, where 1 M KCl extractable acidity is used, and percent organic carbon content (sample kfs3 is off scale: x,y co-ordinates are 8.23, 127).

Saturation indices for the soil solution were calculated with PHREEQC modelling programme (Parkhurst, 1995) utilising the WATEQ4F database (Ball and Nordstrom, 1991) in order to approximate the soil solution - solid phase equilibrium. The pH (H_2O) data were used for

calculating saturation indices rather than pH (SPE) due to the uncertainties associated with measuring pH in the extracts opposed to the saturated pastes themselves. Table 2.3 lists saturation indices for phases most likely controlling Al^{3+} solubility. Due to the uncertainty associated with the Al analysis (ICP-MS which analyses total Al and not strictly monomeric Al) and the use of pH (H_2O) instead of pH (SPE), Table 2.3 is useful only to show approximate trends and not absolute values. The results indicate that at extremely low pH values (pH < 4.3), Al-OM complexes tend to become dominant in controlling Al^{3+} activity instead of gibbsite marked by a higher SI in the former relative to the latter. Fey *et al.* (1998) surmised that acidification resulting from land use practices induces the favourable formation of Al solids of lower basicity at the expense of higher basicity. The authors found that afforestation resulted in an increase in saturation index for solids with lower basicity, specifically Al-OM and jurbanite to a lesser extent, whereas the saturation index of $Al(OH)_3$ (amorphous) tended to decrease.

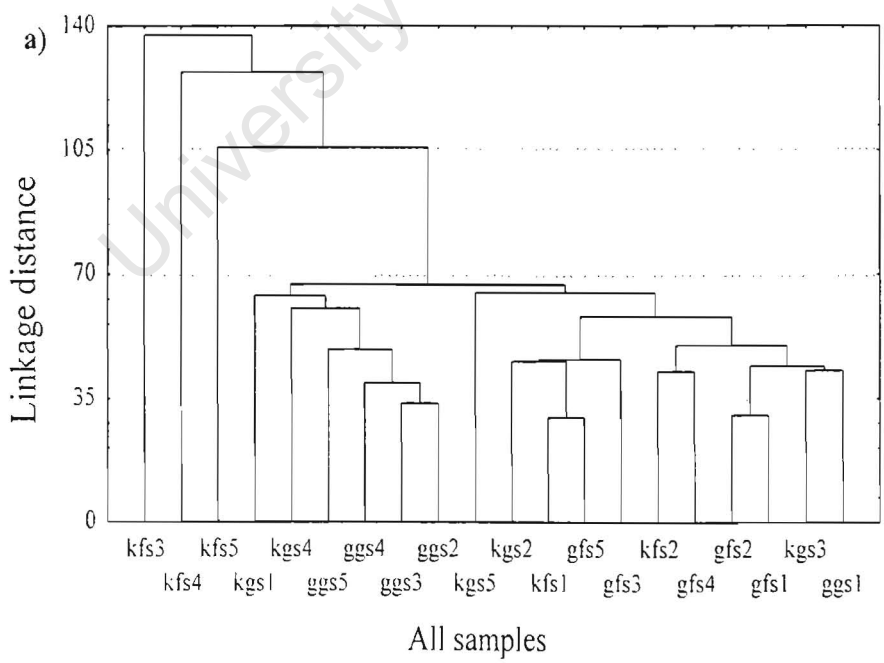
Table 2.3 Saturation indices for selected minerals and pH (H_2O) values for all samples. Highlighted values indicate approximate equilibrium/supersaturation with respect to the corresponding mineral (SI > -0.5).

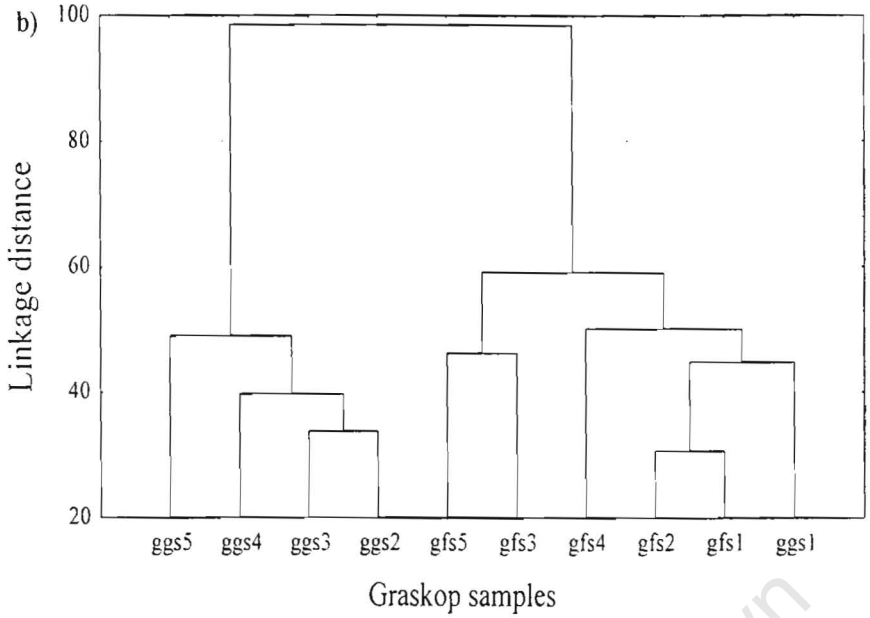
	pH (H_2O)	Gibbsite	Alunite	Al-OM*	Jurbanite
log KT		8.11	-1.40	-2.52	-3.23
ggs1	4.72	-3.28	-9.12	-3.92	-5.49
ggs2	4.86	-1.58	-4.20	-2.74	-3.68
ggs3	4.76	-0.95	-2.29	-1.86	-3.04
ggs4	5.05	**0.72**	**1.96**	-0.92	-1.91
ggs5	5.23	**0.21**	**-0.19**	-1.62	-2.88
gfs1	4.58	-1.09	-2.99	-1.51	-3.11
gfs2	4.69	-3.79	-11.64	-4.45	-6.09
gfs3	4.28	**0.12**	**1.53**	**0.41**	-1.20
gfs4	4.76	-1.57	-5.33	-2.48	-4.05
gfs5	4.47	**0.55**	**2.11**	**0.36**	-1.32
kgs1	5.28	**0.08**	-0.75	-2.03	-3.00
kgs2	5.24	-0.86	-3.96	-2.69	-3.93
kgs3	5.02	-2.68	-9.14	-4.04	-5.60
kgs4	4.55	-2.81	-7.22	-2.95	-4.31
kgs5	4.70	-1.69	-5.23	-2.34	-3.87
kfs1	4.89	-1.69	-5.66	-2.83	-4.27
kfs2	4.68	-3.09	-8.35	-3.75	-4.89
kfs3	3.26	-2.25	-2.08	**0.44**	-1.24
kfs4	4.49	-1.08	-1.88	-1.25	-2.41
kfs5	4.93	**0.64**	**2.31**	-0.25	-1.40

* The equation $\log(Al^{3+}) = -2.52 - 0.6pH$ of Cronan *et al.* (1986) was used for calculation of the solubility product.

2.3.2 *Statistical evaluation of vegetation impacts on soil chemical properties*

The danger of presupposing that observed differences in soils are solely a result of vegetation, rather than of compounded site differences, was recognised. Consequently, following the characterisation of the soils, a cluster analysis was performed to assess the legitimacy of grouping samples according to vegetation for all samples and for samples at both localities (Figure 2.13a, 2.13b and 2.13c). The Graskop samples are well clustered according to vegetation with the exception of one grassland sample, ggs1, whereas the Kaapsehoop samples show no tendency to cluster according to vegetation. The cluster analysis done on all samples from both localities revealed thirteen samples, seven forest and six grassland, for which vegetation is an appropriate grouping variable. These thirteen samples were used in another cluster analysis to confirm their legitimacy in grouping according to vegetation (Figure 2.14). The remaining seven samples were excluded based on confounding site differences. A discussion of this is presented in Appendix C. Statistical assessment regarding impacts of afforestation on soil chemical status was limited to the thirteen samples that cluster according to vegetation.





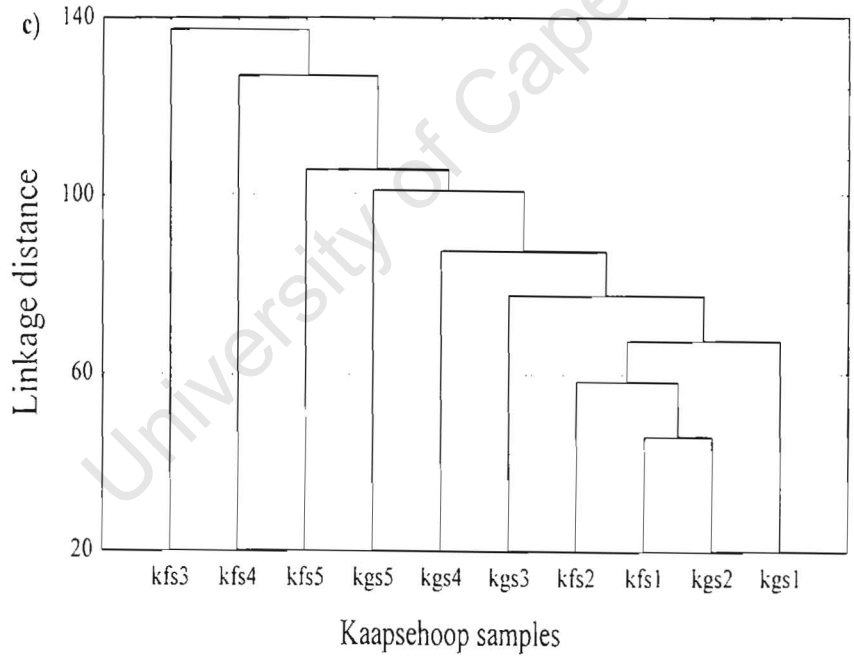


Figure 2.13 Tree diagram cluster analyses illustrating the validity of superimposing vegetation as a grouping variable for a) all samples; b) Graskop samples; c) Kaapsehoop samples, where linkage distance is the Euclidean distance.

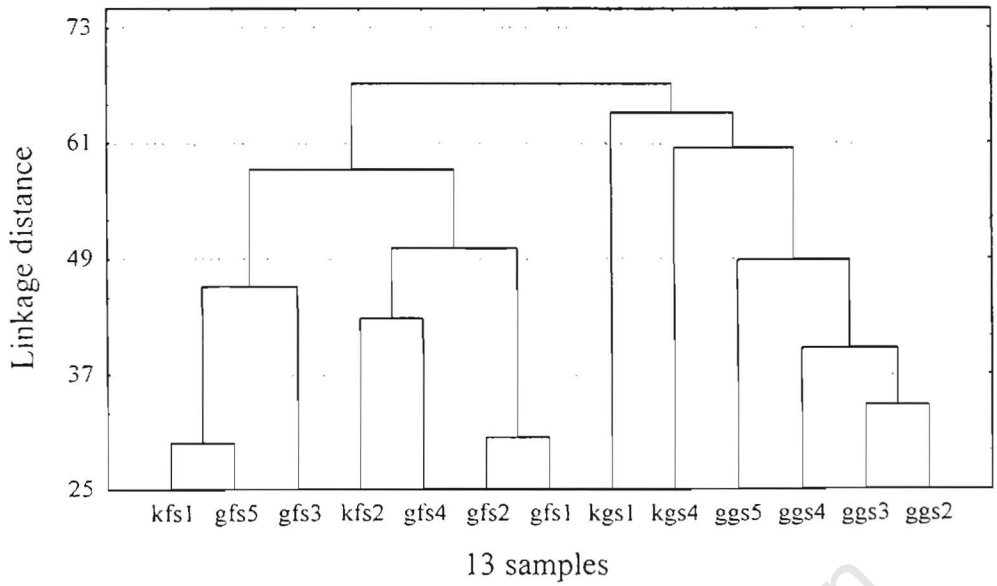


Figure 2.14 Tree diagram cluster analysis of the sub-set of thirteen samples identified in Figure 2.13a, illustrating the validity of grouping samples according to vegetation in order to assess impacts arising from afforestation.

The following section assesses the impact of afforestation on soil chemical properties; the thirteen samples for which vegetation is a legitimate grouping variable are used for the interpretation.

2.3.3 Vegetation impacts on soil chemical properties

2.3.3.1 Acidity status

pH

The difference in pH values between grassland and forest samples is illustrated graphically in Figure 2.15, which shows box and whisker plots of pH (KCl), pH (H_2O) and pH (SPE). In general, the forest samples are characterised by lower pH values than the grassland samples. The median values for the grassland samples are 4.13, 4.96 and 6.16 for pH (KCl), pH (H_2O) and pH (SPE), respectively, whereas the median values for the forest samples are 3.97, 4.68 and 6.01 for the same variables. The difference in pH between the vegetation types is significant at the 72%, 94% and 91% confidence levels for pH (KCl), pH (H_2O) and pH (SPE), respectively. These findings are consistent with previous work assessing acidification resulting from afforestation in the eastern escarpment. Nowicki (1997) reported

p-values < 0.001 for pH (KCl) and pH (H_2O) between thirteen pairs of grassland and forest soil samples in the eastern escarpment. Afforestation-induced acidification appears to be greater in pH (H_2O) data compared to pH (KCl) data (p = 0.06 and p = 0.28, respectively). This may be due, in part, to a decrease in net CEC of the forest samples or a manifestation of AEC due to variably charged exchange sites (see Chapter 3).

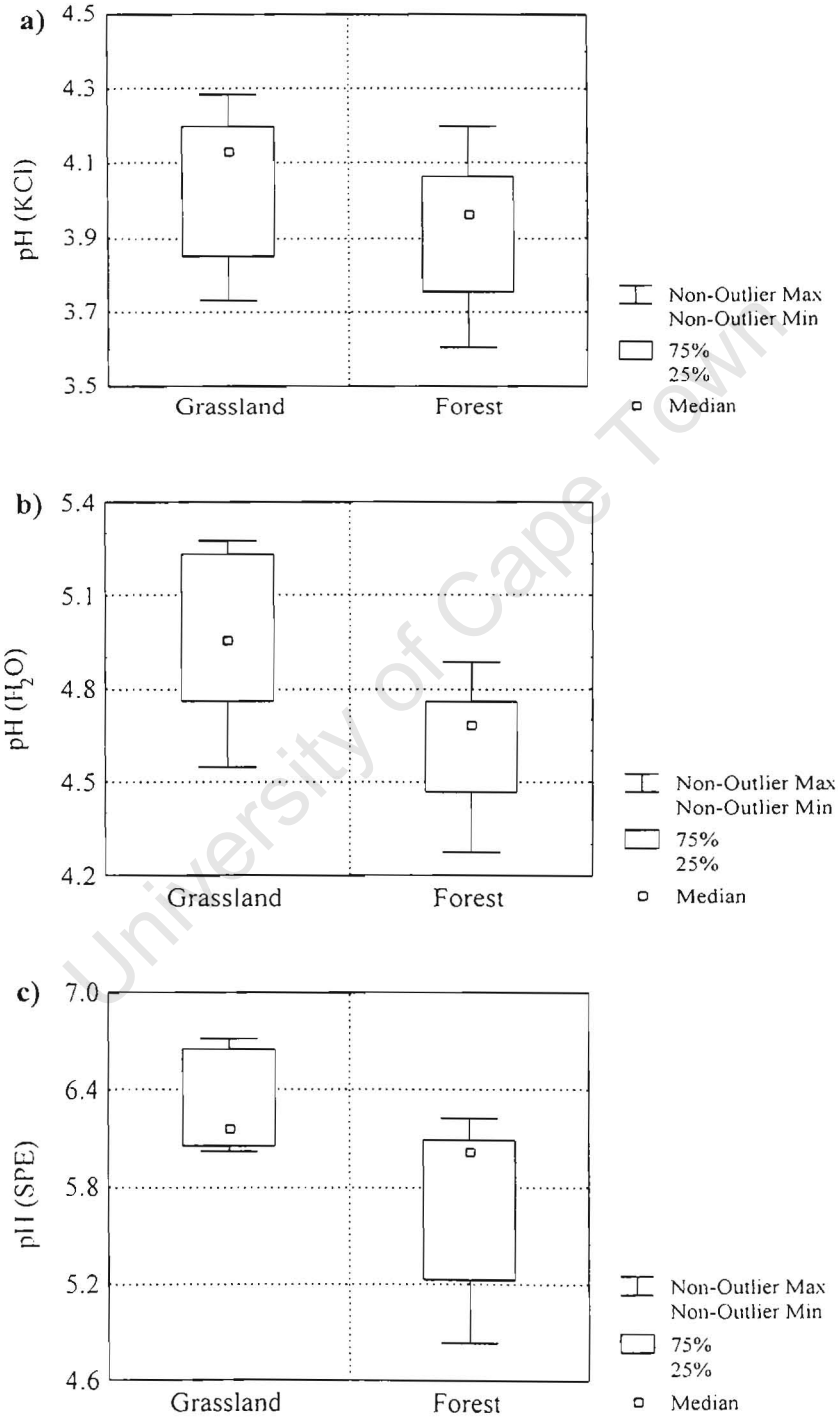


Figure 2.15 Box and whisker plots illustrating differences in pH between grassland and forest samples a) pH (KCl), p = 0.28 b) pH (H_2O), p = 0.06 c) pH (SPE), p = 0.09.

Extractable cations

Extractable cations, basic and acidic, were determined in order to assess acid status of the exchangeable phase of the soil. Figure 2.16 shows a box and whisker plot of total extractable base cations and compares the vegetation types with respect to this parameter. There is an apparent enhancement in total extractable base cations in the forest samples relative to the grassland samples ($p = 0.15$). The median total extractable base cation values are 2.5 and 5.7 $mmol_c kg^{-1}$ for grassland and forest, respectively.

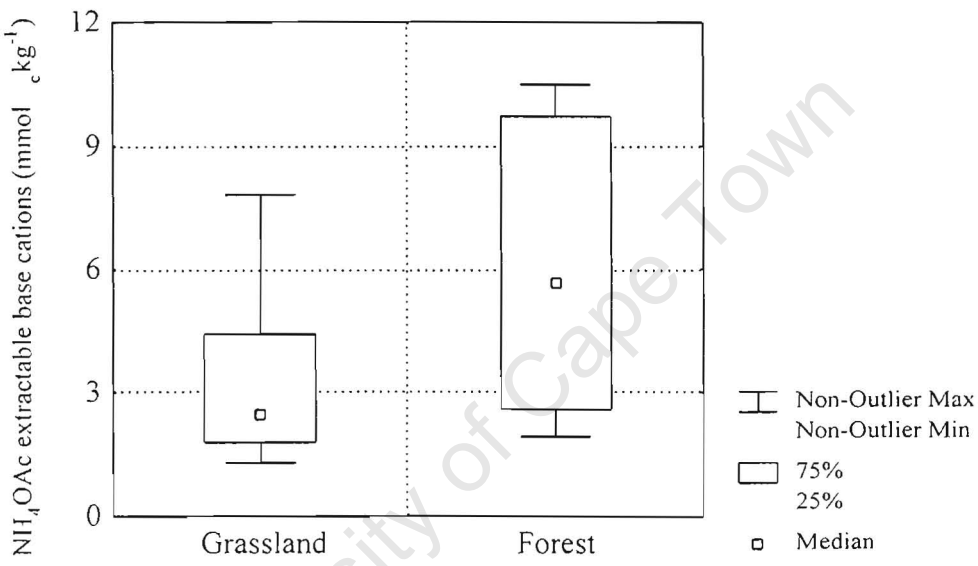


Figure 2.16 Box and whisker plot showing total extractable base cations for grassland and forest samples ($p = 0.15$).

This enhancement of extractable base cations should be interpreted in terms of the total extractable cation suite. Extractable acidity, an aggregate property reflecting acidic exchangeable cations, is shown in Figure 2.17 for the two vegetation types. There is a significant enhancement in extractable acidity in the forest samples relative to the grassland samples, $p = 0.003$.

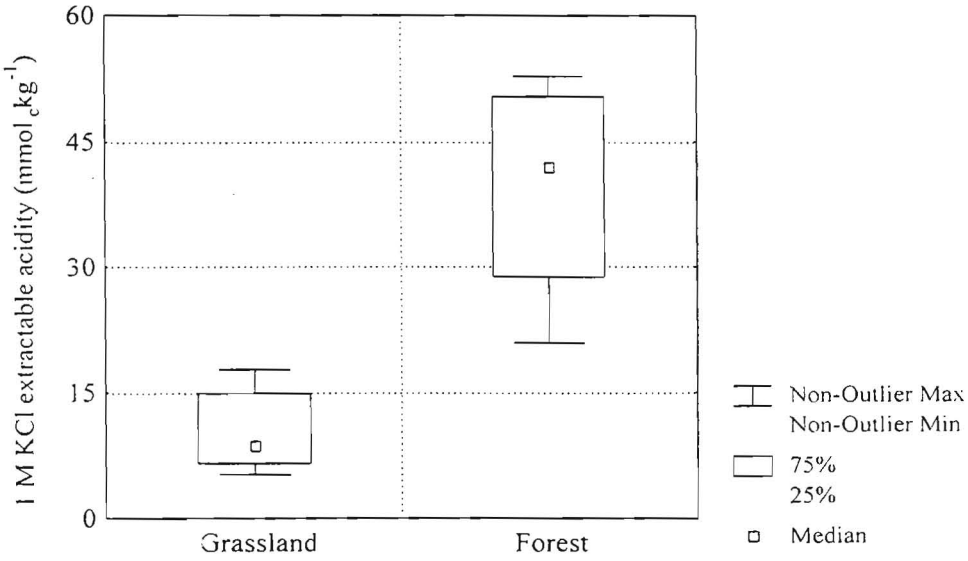


Figure 2.17 Box and whisker plot illustrating the difference in 1 M KCl extractable acidity between vegetation types ($p = 0.003$).

Derived parameters: CEC_e and acid saturation

Effective cation exchange capacity (CEC_e) was calculated as the sum of total extractable base cations and extractable acidity. Table 2.4 lists correlation statistics for selected thirteen samples, grassland samples and forest samples so as to illustrate apparent differences in CEC_e and character of the exchangeable suite of cations associated with the vegetation types. Correlation coefficients reveal a very strong covariance ($r_s = 0.92$, $p < 0.001$) between CEC_e and organic carbon, and a slightly less strong between CEC_e and clay content ($r_s = 0.65$, $p = 0.02$) for all the samples. Examination of the vegetation types as exclusive sub-groups shows a strong correlation between organic carbon and CEC_e for the forest samples ($r_s = 0.89$, $p = 0.007$), whereas the CEC_e of the grassland samples is better correlated with clay content ($r_s = 0.77$, $p = 0.08$). Figure 2.18 shows the significant correlation between CEC_e and organic carbon content. This significant correlation, particularly for the forest samples, suggests that the CEC of the soils can be attributed to organic matter that carries negative surface charge. There is a clear grouping according to vegetation in the plot revealing significantly more organic carbon in the forest samples ($p = 0.004$), which in turn leads to the enhancement of CEC_e ($p = 0.003$). This is consistent with detailed studies reviewed by Sposito (1989) and McColl and Gressel (1995) which report a positive correlation between cation exchange capacity and soil organic matter content.

Table 2.4 Spearman's correlation coefficient (r_s) for all samples, forest samples and grassland samples for selected variables illustrating differences in exchangeable cation suite between the vegetation types.

	All samples n = 13	**Forest** n = 7	**Grassland** n = 6
CEC_e and organic carbon	0.92***	0.89**	0.54
CEC_e and clay content	0.65*	-0.24	0.77
Extractable acidity and organic carbon	0.90***	0.82*	0.37
Extractable base cations and organic carbon	0.70**	0.71	0.71
Extractable acidity and clay content	0.59*	-0.38	0.54
Extractable base cations and clay content	0.69**	0.36	1.00***

*, **, *** – Significant at the 95%, 99% and 99.9% confidence level, respectively

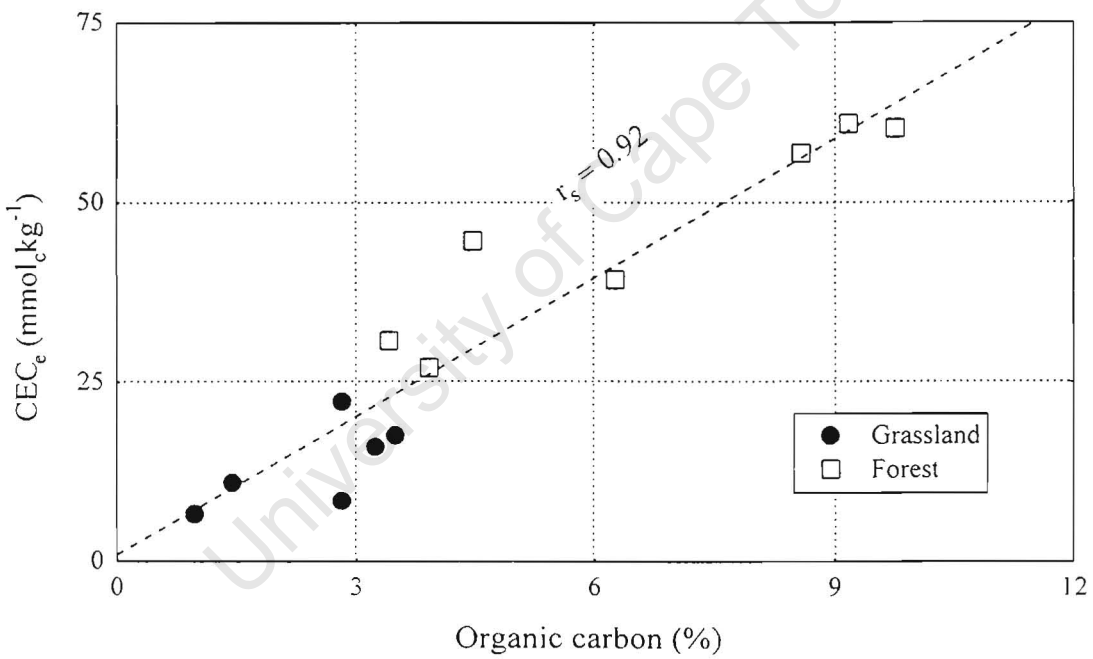


Figure 2.18 The relationship between CEC_e and percent organic carbon content relative to vegetation type ($r_s = 0.92$, $p < 0.001$).

It has been established that there is a substantially larger CEC_e associated with the forest samples compared to the grassland samples, evident through the enhancement of *both* total extractable base cations and extractable acidity. The acidic character of the exchangeable suite of cations is examined in Figure 2.19, in which extractable acidity is plotted against organic carbon. As suggested by Table 2.4, there are distinct correlations between the two variables according to vegetation type. Figure 2.19 shows a clear distinction between the

vegetation types, suggesting that not only is there a significant difference in organic matter content, but there is also a distinction in organic matter quality between the vegetation types. Any given amount of organic matter is expected to have an enhanced Al or acidic status in the forest samples relative to grassland samples. Soil organic matter is known to play a major role in buffering protons and metal cation concentrations in soil solution by way of cation exchange (Sposito, 1989). This observation is well illustrated in Figure 2.20 which shows acid saturation, the relative proportion of exchange sites occupied by acidic cations, for the two vegetation types. There is an enhancement of acid saturation in the forest samples compared to grassland samples significant at the 95% confidence level.

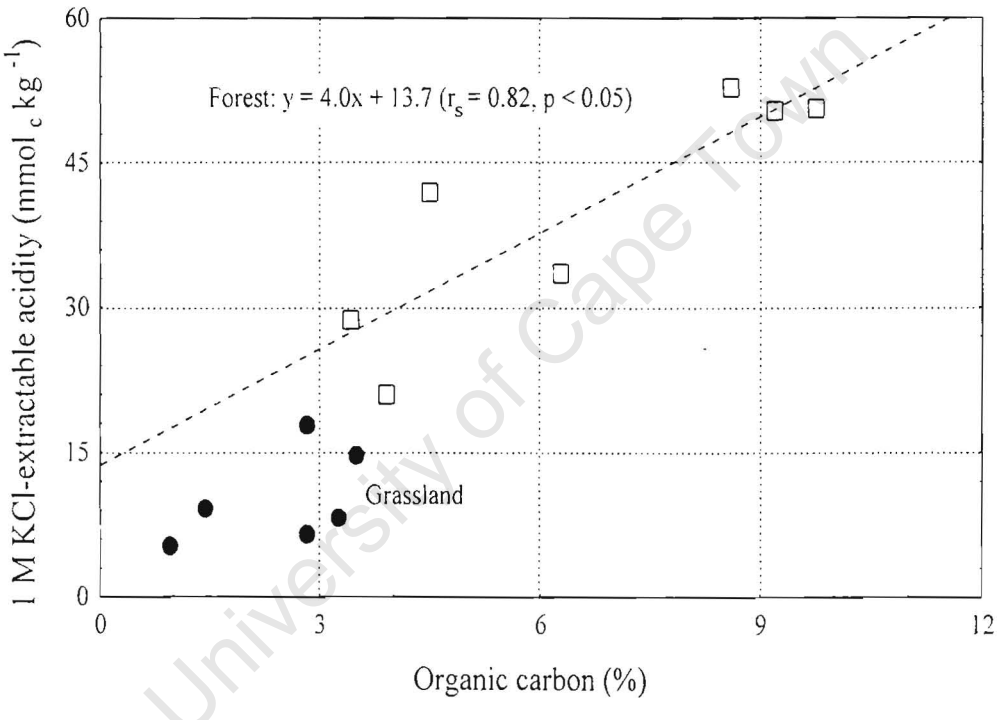


Figure 2.19 The relationship between extractable acidity and percent organic carbon content illustrating a clear distinction between vegetation types.

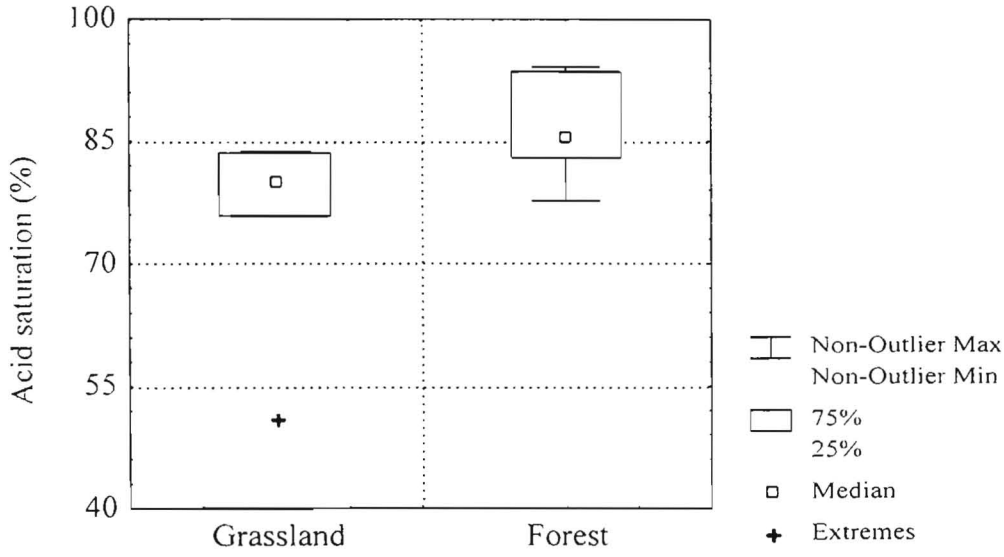


Figure 2.20 Box and whisker plot illustrating the difference in acid saturation between vegetation types (p = 0.03).

2.3.3.2 Electrical conductivity

The EC of the soil solution reflects to a large extent the ionic strength of the solution. An interesting distinction between grassland and forest samples arises with respect to this parameter. There is significantly higher EC in the grassland samples relative to the forest samples. The median values of EC are 278 and 201 μScm^{-1} for the grassland and forest samples, respectively. This difference is significant at the 99% confidence level as illustrated in the box and whisker pot in Figure 2.21. This difference is contrary to what Nowicki (1997) found in his study of grassland and forest samples in the eastern escarpment, which revealed an enhanced EC in the forest samples significant at the 95% confidence level. This discrepancy can be attributed to the different sampling strategy used for the two studies. The current study included the partially decomposed litter layer in the forest samples, whereas the previous study did not. The inclusion of the litter layer has resulted in a significant enhancement of organic matter in the forest samples, which in turn leads to an enhanced cation exchange capacity. The enhanced cation exchange capacity of the forest samples in this study may explain, in part, the apparent depletion of EC. A large cation exchange capacity results from a soil containing a large number of sorption sites. Cations are expected to be less mobile in these environments as they will be bound to exchange sites. Soluble and strongly hydrating cations (namely, Na^+, K^+, Ca^{2+}, and Mg^{2+}), are strongly held by humus clays and are, therefore, expected to be relatively more concentrated in the soil solution of soils with lower CEC (McBride, 1994) resulting in an enhancement of EC. This is evident in

the separation between vegetation types when EC is plotted against CEC_e as seen in Figure 2.22, which shows the forest samples characterised by low EC and high CEC_e whereas the grassland samples are characterised by high EC and low CEC_e. There is a general negative correlation between the two variables, substantiating the link between relatively enhanced CEC_e and reduced EC (r_s = -0.60 and p = 0.03).

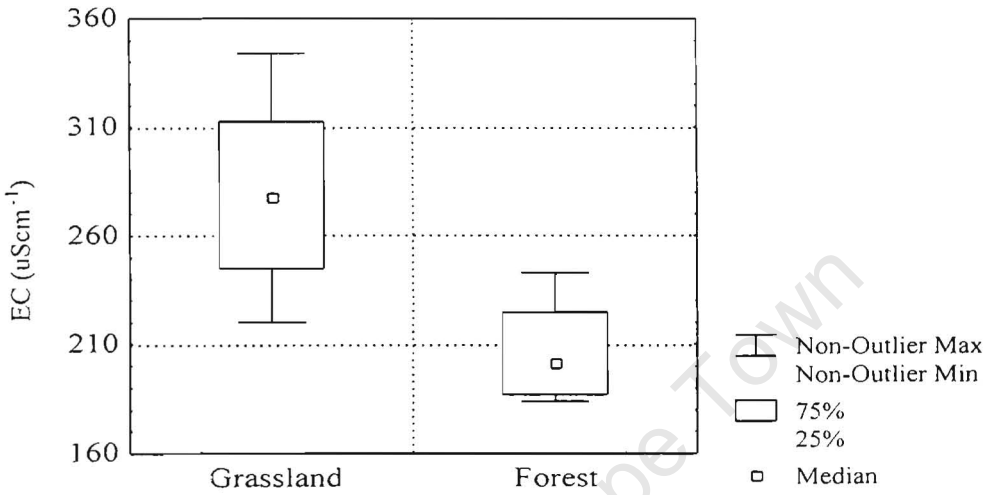


Figure 2.21 Box and whisker plot of illustrating the difference in electrical conductivity between vegetation types (p = 0.007).

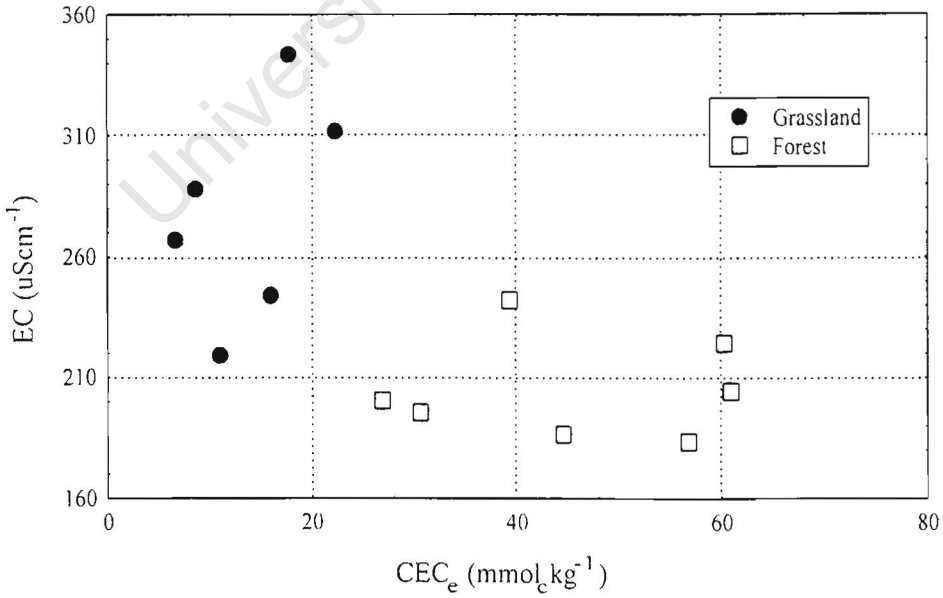


Figure 2.22 Bivariate plot of electrical conductivity of the saturated paste extracts and effective cation exchange capacity, in relation to vegetation types (r_s = -0.60, p = 0.03).

2.3.3.3 Nitrogen status

The N status of the soil samples was evaluated with respect to total N, C/N ratio, soil solution mineral N (NO_3^- and NH_4^+) and 2 M KCl-extractable mineral N (NO_3^- and NH_4^+).

Total N and C/N ratio

Total N was determined with the Kjeldahl digestion method that consists of the oxidation of organic N to NH_4^+-N followed by the quantitative colorimetric determination of NH_4^+-N. Due to the small fraction of total N constituted by mineral N (0.2 - 2.4%), the total N was taken as a reasonable estimate of organic N. A box and whisker plot of total N (Figure 2.23) shows that there is a significant difference in total N values between the forest and grassland samples. The median values of total N are 0.14 and 0.33% for the grassland and forest samples, respectively.

Similarly to total N levels, organic C content determined by wet oxidation, is enhanced in the forest relative to the grassland soils. The median values for organic C are 2.8% for grassland and 6.3% for forest soils reflecting an enhancement in the forest soils significant at the 99% confidence level (Figure 2.24). The enhancement of organic C, reflecting a higher organic matter status, is mostly attributed to the inclusion of the partially decomposed litter layer in the forest samples.

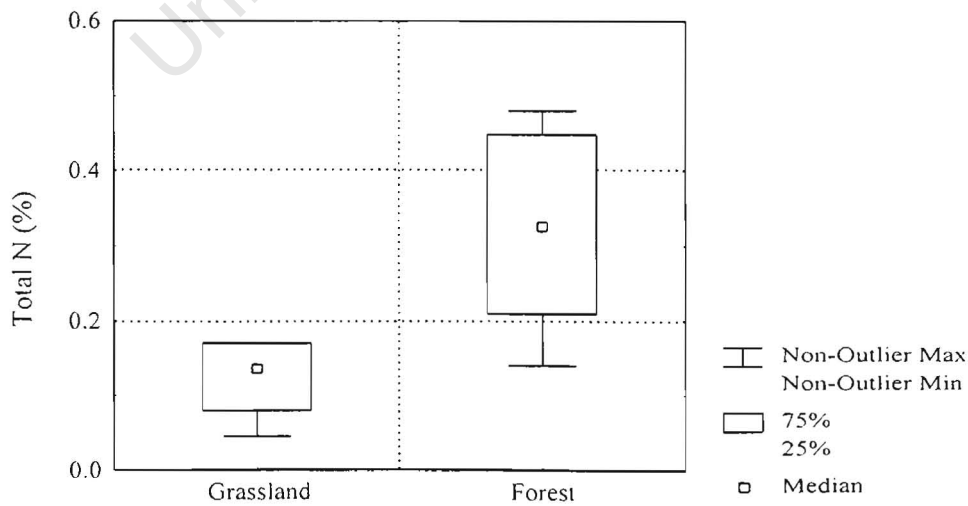


Figure 2.23 Box and whisker plot illustrating the significant difference in percent total N between vegetation types (p = 0.010).

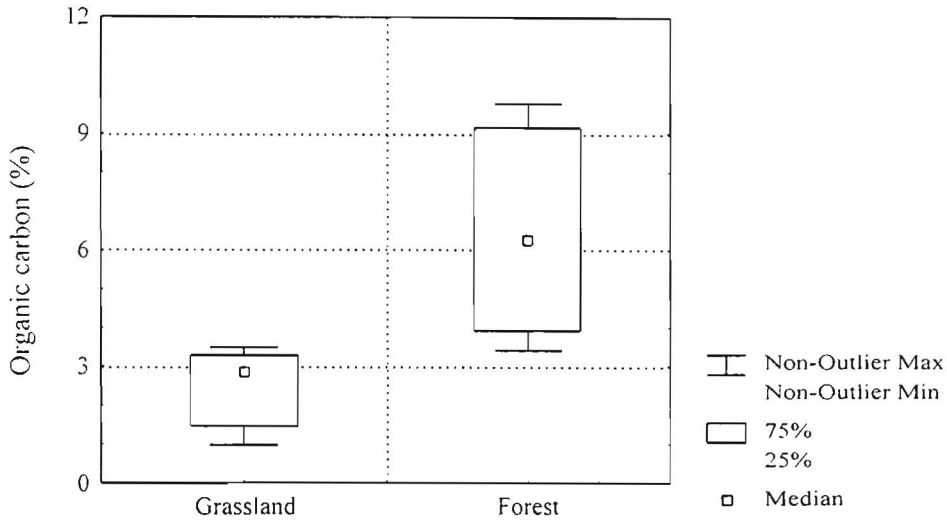


Figure 2.24 Box and whisker plot illustrating the significant difference in percent organic carbon between vegetation types (p = 0.004).

The enhanced total N status of the forest soils is attributed to the enhanced organic matter status since the largest pool of soil N is organic matter and the relative enhancement from grassland to forest is of similar magnitude (approximately doubled) for both parameters. This is corroborated by the highly significant correlation between organic C and total N for which both grassland and forest samples follow the same trend (Figure 2.25). A ratio of organic C percent and total N percent was used to estimate the C/N ratios for the samples. As expected from the singular trend between organic C and total N for both grassland and forest samples, there is no significant difference in C/N between vegetation types (Figure 2.26). The median value of C/N is 20 for both grassland and forest and the range is 18-24 and 18-26 for grassland and forest samples, respectively. These C/N ratios are similar to those which Gundersen *et al.* (1998) reported in organic layers in forest soils of high N status, with values varying from about 20 for high N status sites to 33 for low N status sites.

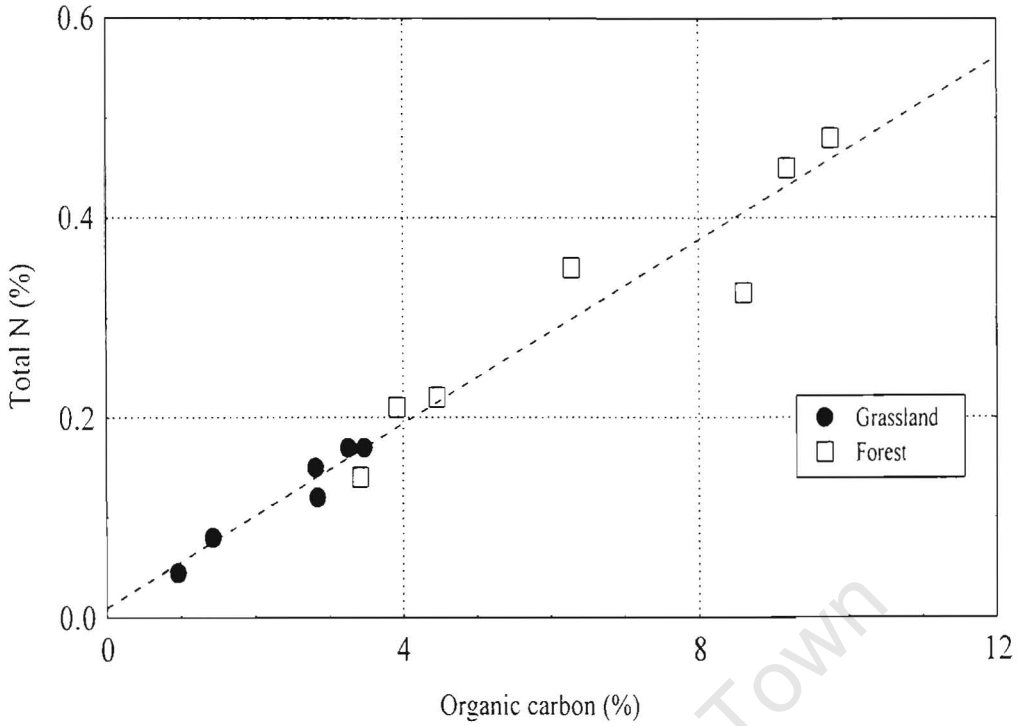


Figure 2.25 The significant correlation between percent total N and percent organic carbon for all thirteen samples regardless of vegetation type ($r_s = 0.96$, $p < 0.001$).

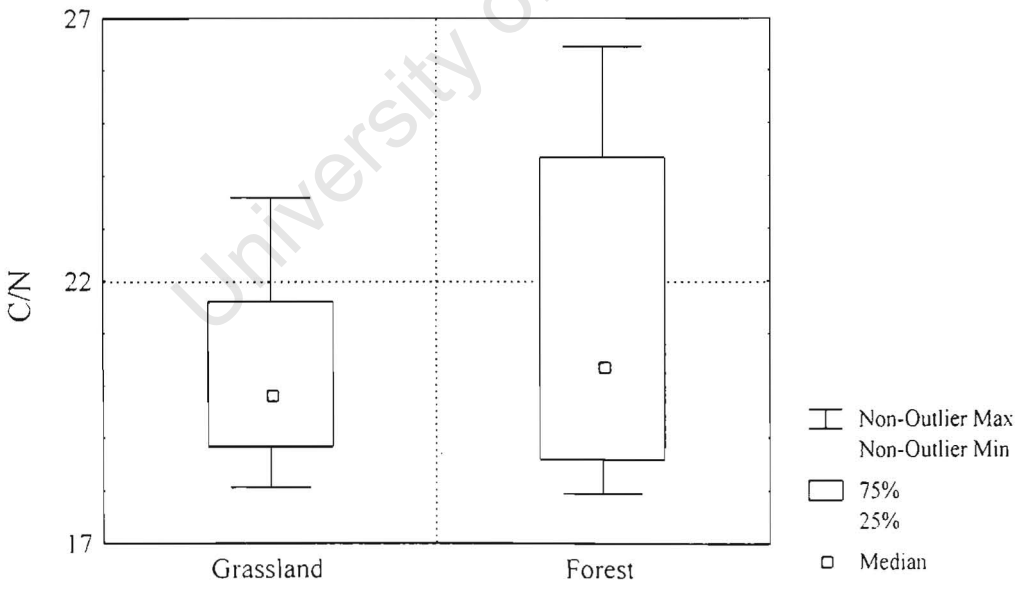


Figure 2.26 Box and whisker plot illustrating the similarity in C/N between vegetation types ($p = 0.886$).

Much current research has focused on the correlation between C/N ratio and mineralisation and nitrification rates, NO_3 leaching and N status in afforested areas around the world (Van Miegroet *et al.*, 1989; Emmett *et al.*, 1998; Gundersen *et al.*, 1998). The substantiated link

between the parameters relating to N status and C/N is, however, only consistent for C/N in the forest floor and not in the mineral soil. Gundersen *et al.* (1998) reported, in a synthesis of the NITREX project, that mineral soil C/N is not correlated to mineralisation and the mineral pool might not need to change for N saturation to occur. The implications for this study are that the combining of organic forest floor soil horizons and the mineral horizon in the forest samples might have obscured differences in mineralisation and N status that may have otherwise been apparent had the horizons been sampled separately. As it is, however, the similarity of the C/N between vegetation types in these samples is critical in assessing any differences observed in mineral N status and/or mineralisation rates (Chapter 3). Any observed differences in mineralisation cannot be attributed to an enhanced nitrogen status relative to the organic C, but rather to organic matter quality, inasmuch as can be inferred regarding the proportion of the pool that is mineralisable and that which is refractory. This is considered at length in Chapter 3 when discussing the results of the mineralisation experiments.

Soil solution mineral N

Major ions in the saturated paste extracts were determined with ion chromatography including NH_4^+, NO_2^- and NO_3^-. Nitrite concentrations were below detection limits for all 20 original samples and, therefore, considered negligible to the soluble and total mineral N pool. This is common, as NO_2^- is generally a transitory form of soil N in oxidation and reduction reactions (Harris, 1988). There are well-documented difficulties in assessing mineral N status in saturated paste extracts due to the re-wetting of air-dried samples which can result in a burst of microbial activity, particularly leading to a pulse of nitrification (Bartlett and James, 1980; Bartlett, 1986). In retrospect, refrigerated field-moist samples should have been used in the preparation of saturated paste extracts. Since, however, the primary purpose of this study is to compare N status between grassland and forest samples the largest mitigating factor is consistency of methods between samples in order to justifiably assess these differences. Although the absolute values determined for mineral N status may be somewhat uncertain, the comparison between vegetation types should still serve its purpose, namely to elucidate differences in mineral N status arising from afforestation.

Soil solution NO_3^- concentrations are presented in the box and whisker plot in Figure 2.27, which shows no significant difference in median value between vegetation types ($p = 0.57$). The median values of NO_3^- in the soil solutions were 0.057 and 0.075 mmol l^{-1} for the

grassland and forest samples, respectively. While assessing NO_3^- in solution, it may be important to consider not only the absolute concentrations, but also the contribution the constituent makes to the ionic strength of the soil solution. As discussed in section 2.4.3.2, the grassland soil solution is characterised by a significantly higher EC than the forest samples ($p = 0.007$). In order to assess NO_3^- concentration relative to the other ions in solution, it was necessary to normalise the results with respect to ionic strength[2], using a simple ratio hereafter referred to as NO_3^-/I. The median values of NO_3^-/I are 0.023 and 0.038 for grassland and forest samples, respectively. Normalising with respect to ionic strength reveals a relative enhancement of NO_3^- in the forest samples compared to grassland soils, significant at the 80% confidence level.

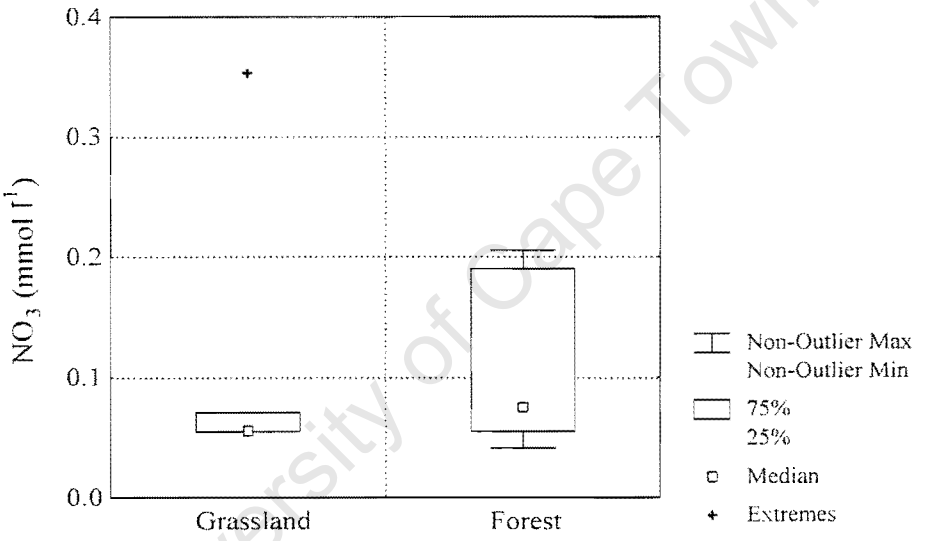


Figure 2.27 Box and whisker plot illustrating soil solution NO_3^- (mmol l^{-1}) between the vegetation types ($p = 0.57$).

The water-soluble NO_3^- status of the soil can also be assessed in terms of the amount of NO_3^- relative to the dry mass of the soil. This perspective interprets the saturated paste procedure as an extraction technique. Due to differences in physical soil properties, particularly texture, the amount of water used for the extraction varied substantially between samples. Expressing soluble NO_3^- in terms of dry mass of soil gives the following median values: 0.019 and 0.053 mmol kg^{-1} for grassland and forest samples, respectively. An enhancement of NO_3^- in the forest samples now becomes evident, significant at the 88% confidence level. As mentioned, this enhancement is probably due to differences in texture, the forest samples being

[2] $I = \frac{1}{2}\Sigma c_i z_i^2$, where c_i = molar concentration of the i^{th} ion in mmol l^{-1} and z_i = the charge on the i^{th} ion

characterised by a higher clay content relative to the grassland samples. A finer textured soil generally necessitates a larger proportion of water to produce a saturated paste. The median values of clay content for the samples are 2.2% for grassland and 4.3% for forest ($p = 0.02$). It may also be useful to interpret the amount of NO_3^- per dry mass of soil relative to the total suite of water-soluble ions. If a soil solution is concentrated through evapotranspiration, it is expected that all of the conservative ions not influenced by precipitation and dissolution reactions will be concentrated accordingly. Since the grassland soil solutions have a stronger ionic strength, due to more limited availability of exchange sites, it is necessary when comparing concentrations of any one ion between vegetation types to take this into consideration. It follows that normalising with respect to ionic strength (where ionic strength is calculated from the moles of ions per mass of soil[3]) will provide a more appropriate means by which a meaningful comparison between vegetation types with respect to water-soluble NO_3^- can be made. Figure 2.28 shows the comparison between vegetation types for NO_3^- per kg of soil normalised with respect to ionic strength. This comparison reveals an enhancement of NO_3^- status in the forest (median = 0.06) relative to the grassland samples (median = 0.02), $p = 0.03$.

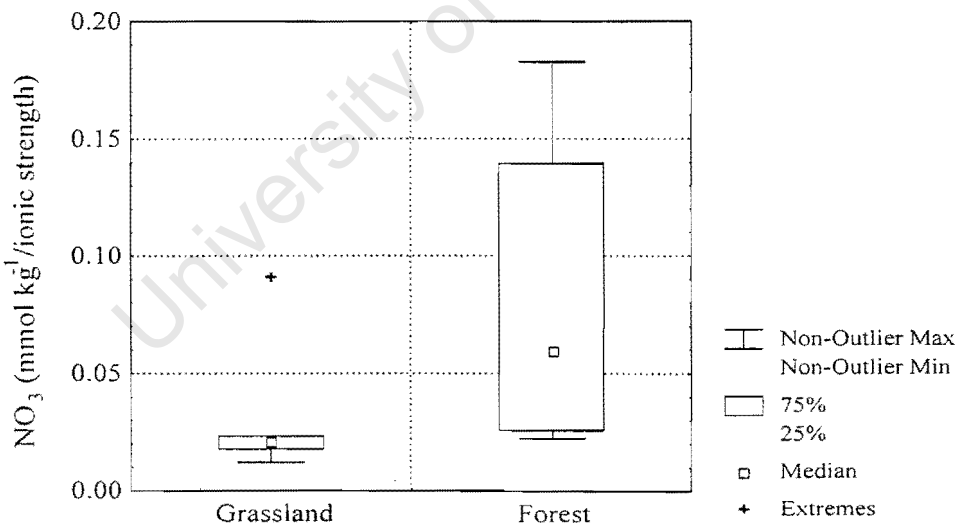


Figure 2.28 Box and whisker plot comparing soil solution NO_3^- per mass of dry soil normalised with respect to ionic strength between the vegetation types ($p = 0.03$).

It transpires that the forest samples are significantly enhanced in water-soluble NO_3^- relative to the grassland samples once ionic strength is considered. Despite the larger amount of NO_3^-

[3] $I = \frac{1}{2}\Sigma c_i z_i^2$, where c_i = molar concentration of the i^{th} ion in mmol kg^{-1} and z_i = the charge on the i^{th} ion

in the forest relative to grassland soil solution, in terms of absolute values there is nothing alarming about these NO_3^- levels, which in terms of water quality criteria are unlikely to pose an environmental threat to the ecosystem.

Similar reasoning to that described above was employed to interpret water-soluble NH_4^+ in the soil samples. Firstly, molar concentrations of NH_4^+ in the soil solution were compared between vegetation types (Figure 2.29). The median values are 0.16 and 0.09 mmol l^{-1} in the grassland and forest samples, respectively, translating to an enhancement in the grassland samples relative to forest ($p = 0.07$). Normalising with respect to ionic strength would elevate the NH_4^+ status in the forest samples relative to the grassland samples, as the forest samples are less saline.

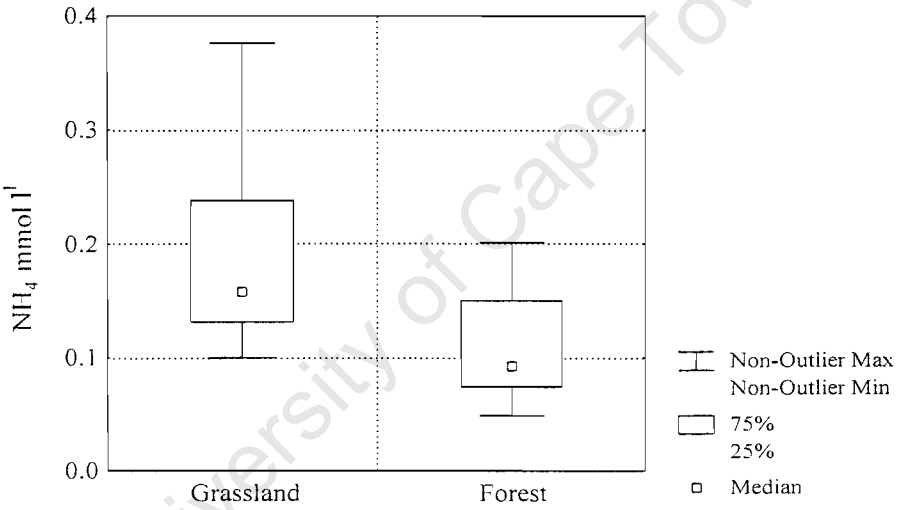


Figure 2.29 Box and whisker plot illustrating the difference in soil solution NH_4^+ concentration between the vegetation types ($p = 0.07$).

Ammonium status in the soil solution was also interpreted relative to dry mass of soil. It follows, as was the case with NO_3^-, that since the forest samples are characterised by a finer texture, expressing NH_4^+ per mass of soil will also reveal an apparent enhancement in the forest samples relative to the grassland. Completing the interpretation, NH_4^+ per mass of soil was normalised with respect to ionic strength (as previously described) revealing a slight further enhancement of NH_4^+ in the forest samples relative to grassland. This is not, however, significant ($p = 0.39$; Figure 2.30).

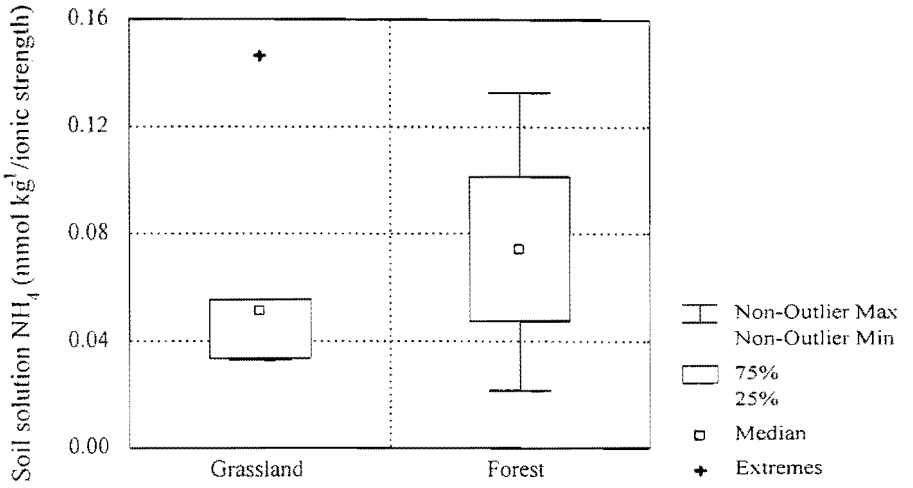


Figure 2.30 Box and whisker plot comparing soil solution NH_4^+ per mass of dry soil normalised with respect to ionic strength between vegetation types (p = 0.39).

2 M KCl-extractable NH_4^+ and NO_3^-

Extractable NH_4^+ and NO_3^- were determined by colorimetry in 2 M KCl extracts. A 2 M solution was used primarily for two reasons, to provide sufficient ions, K^+ and Cl^-, to produce a mass action effect to remove NH_4^+ and NO_3^- associated with exchange sites, and to provide a significant osmotic potential to inhibit further microbial transformation (Stock, 1983). There is a significant enrichment in extractable NO_3^- concentration in the forest samples relative to grassland samples. The median values of extractable NO_3^- are 0.15 and 0.28 mmol kg^{-1} for the grassland and forest samples, respectively, a difference significant at the 94% confidence level (p = 0.06; Figure 2.31). Similarly, there is a relative, but less significant, enhancement in extractable NH_4^+ concentration in the forest samples. The median values for extractable NH_4^+ are 0.26 and 0.45 mmol kg^{-1} for the grassland and forest samples, respectively, significant at the 85% confidence level (p = 0.15; Figure 2.32).

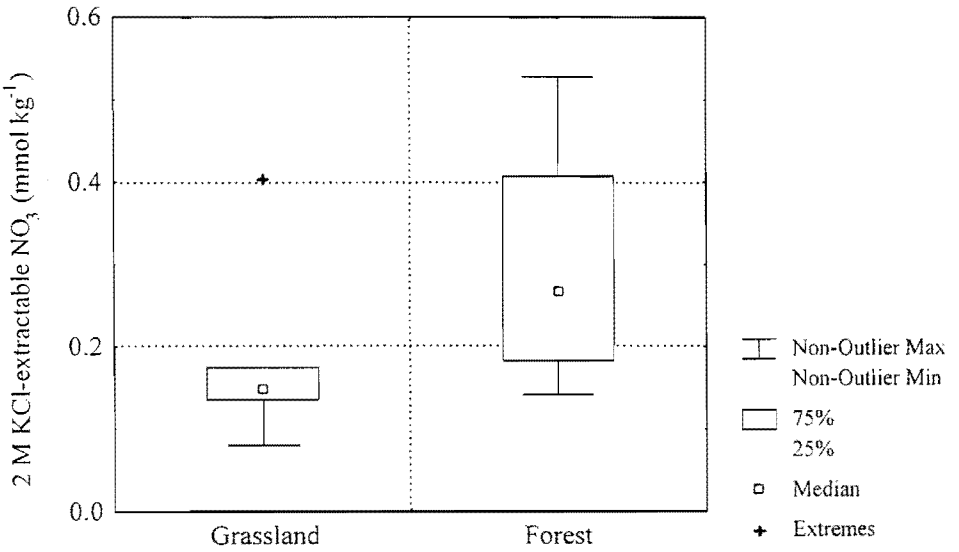


Figure 2.31 Box and whisker plot illustrating the difference in 2 M KCl-extractable NO_3^- (mmol kg^{-1}) between vegetation types (p = 0.06).

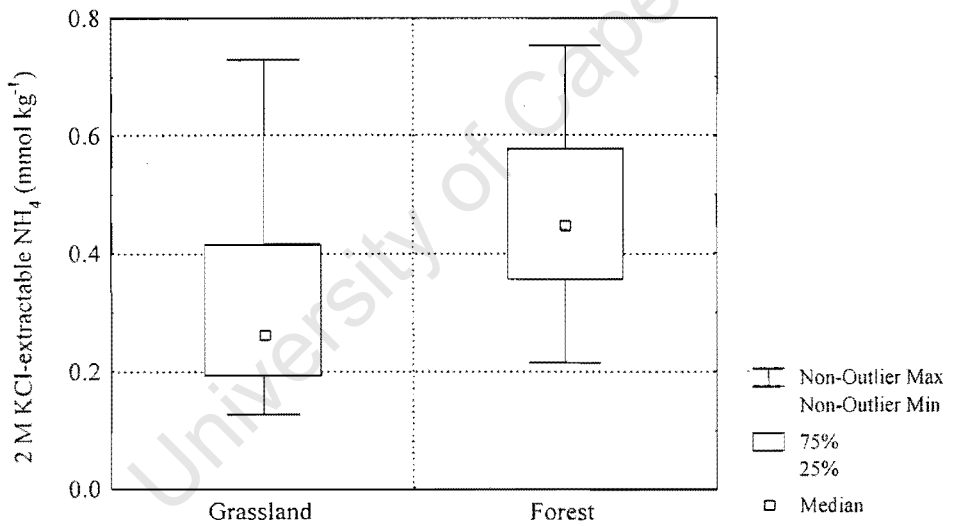


Figure 2.32 Box and whisker plot comparing 2 M KCl-extractable NH_4^+ (mmol kg^{-1}) between the vegetation types (p = 0.15).

In order to assess differences in soil solution and extractable NO_3^-, the two variables were plotted in Figure 2.33. It is apparent from the shallow slope (<1) of the trendline that there is substantially more NO_3^- in the 2 M KCl extracts than in the soil solution for all of the samples. A number of factors may contribute to this difference. The apparent enhancement of NO_3^- in the extracts may be caused by an anion exchange capacity in these acidic topsoils. Particularly, variably charged hydroxyl groups associated with organic matter, which under acidic conditions may become protonated or complexed with metals (namely Al), may carry a

partial positive charge inducing an anion exchange capacity (Chapter 3). There are, however, other factors to consider. The soil : extract ratios for the two techniques were different: the saturated paste extracts had a ratio of ~ 1:0.5, whereas the 2 M KCl extraction was done in a ratio of 1:10. There consequently may be a dilution factor to consider (Chapter 3). It is also possible that the osmotic potential of the 2 M KCl solution destroyed the cells of the nitrogen-transforming microbes, resulting in a release of NO_3^-. Another possibility is that the equilibration of the saturated pastes (24 hours) induced reducing conditions causing the soils to denitrify. Another factor to consider is that the saturated paste extracts were made with air-dried samples, whereas the KCl extracts were made with the field-moist refrigerated samples. It is possible to imagine a pulse of nitrification caused by the re-wetting of the air-dried sample followed by intense denitrification induced by the reducing conditions stemming from the saturation of the samples. Bartlett and James (1980) report that flooding soils that had been dried for storage intensifies development of anaerobic conditions and enhances the rate of denitrification due to reduced organic matter made more available by drying. These authors also reported considerably higher emission of N_2O from remoistened air-dried soils than for continually moist soils. Suffice it to say, the number of permutations of the above-mentioned factors, which is not necessarily an exhaustive list, attests to the complexity of interpreting the apparent enhancement of NO_3^- in the KCl extracts relative to the soil solutions.

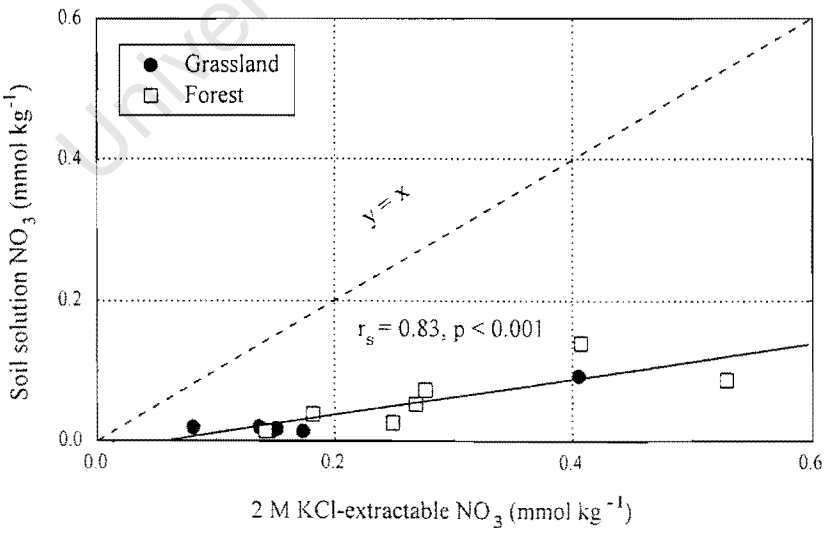


Figure 2.33 The relationship between soil solution NO_3^- and 2 M KCl-extractable NO_3^-, reference line y = x illustrates a shallow slope for trend line: y = 0.26x – 0.01.

A similar comparison of soil solution and extractable concentrations is made for NH_4^+ in Figure 2.34. As with NO_3^-, there is a substantial enhancement in NH_4^+ concentration in the KCl extracts relative to the soil solutions. This apparent enhancement may primarily be attributed to cation exchange capacity. The mass action effect of the K^+ ions would be expected to displace any NH_4^+ ions associated with exchange sites. As discussed for NO_3^-, however, there are many other possible factors contributing to the observation, namely, varying soil: extract ratios and the use of air-dried opposed to field-moist samples, which may have induced nitrification of the NH_4^+ followed by denitrification of the NO_3^- in the saturated paste extracts (Bartlett, 1986; Bartlett and James 1980).

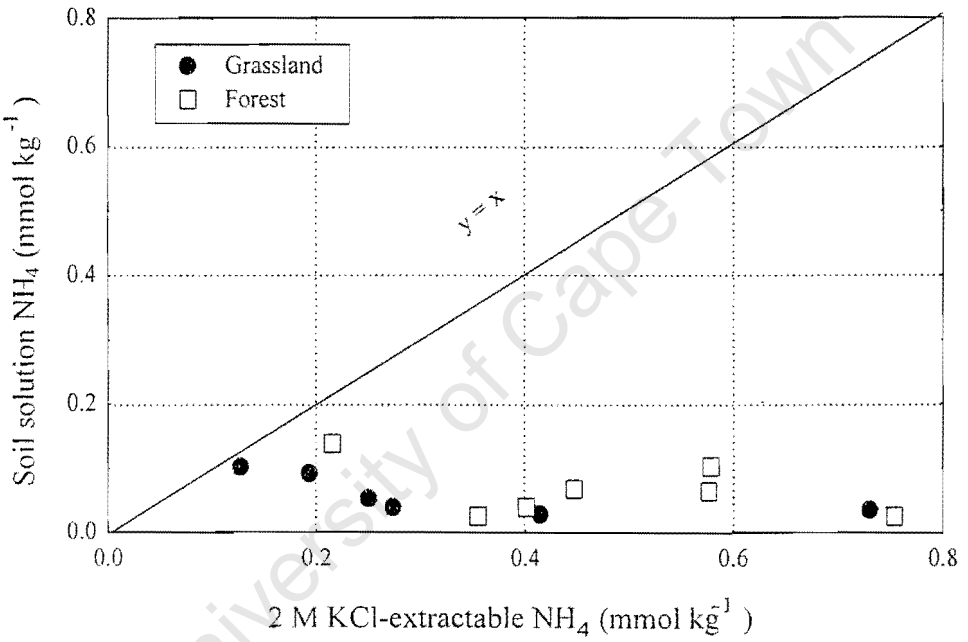


Figure 2.34 The relationship between soil solution NH_4^+ and 2 M KCl-extractable NH_4^+, reference line: y = x.

2.4 General discussion and conclusions

General chemical and physical assessment of the soil samples has demonstrated a consistently highly leached, clay-poor, humus-rich acidic nature of all the soils. The soil solution ionic composition is dominated by Na^+, K^+ and Cl^- and is characterised by low ionic strength. Divalent cations (Ca^{2+} and Mg^{2+}) dominate the extractable base cation suite, complementing the monovalent ion dominance in the soil solution. The dominance of acidic cations in the extractable cation suite is pronounced, with approximately four times greater acidity relative to the total extractable base cations. The enhancement of extractable acidity translates into a

median acid saturation of 82%, approaching the 85% threshold reported for acid stress for acid-tolerant vegetation (Ulrich, 1991). The soils are characterised by generally low CEC_e, reflecting a highly leached status, which is correlated significantly to organic C content. Aluminium speciation and solubility, although limited due to the exclusion of DOC analysis and the analytical techniques employed, reveals a possible tendency for the prevalence of solid Al-OM complexes governing Al solubility at very low pH.

Cluster analyses revealed thirteen suitable samples (six grassland and seven forest) for which vegetation was a legitimate grouping variable. These in turn were used for interpreting differences in soil chemical properties arising from differences in vegetation. Afforestation-induced acidification is apparent in reduced pH, enhanced extractable acidity and enhanced acid saturation in the forest samples relative to the grassland samples. The forest samples are characterised by significantly higher CEC_e relative to the grassland samples, which has been attributed to the inclusion of forest floor organic horizons in the sampling resulting in enhanced organic matter content. Organic matter in the forest samples may contribute significantly to the buffer capacity of the soils, as is apparent from the enhanced acidity status for given organic matter content in the forest samples relative to the grassland samples. Not only is there an enhanced organic matter content in the forest samples relative to the grassland samples, but there may also be a distinction in organic matter quality with respect to acidic character. The enhanced organic matter content in the forest samples indirectly influences the relative lower EC in the forest samples, since there is an apparent negative correlation between CEC_e and EC implying that the enhanced CEC_e in the forest samples, due largely to organic matter content, is responsible for the consistently low EC relative to the grassland samples.

The impact of afforestation on N status of the soils was evaluated in terms of total N, C/N, soil solution mineral N (NO_3^- and NH_4^+) and 2 M KCl extractable mineral N (NO_3^- and NH_4^+). There is a higher total N status in the forest samples relative to the grassland samples reflecting the higher organic C content in the former relative to the latter. Both organic C and total N content is approximately doubled in the forest samples relative to the grassland samples, indicative of the significant correlation between the two parameters. Despite the enhanced total N status of the forest samples, there is no difference in C/N between vegetation types. Interpretation of N status based on C/N requires caution since the forest samples included organic forest floor as well as the top mineral soil horizons. Current research suggests that while C/N in the forest floor is well correlated with overall N status, C/N in the

mineral soil is not. The combination of the organic and mineral horizons in the forest samples may have obscured differences in N status that may otherwise have been apparent had the sampling methodology been different.

Upon first glance there does not appear to be any significant difference in soil solution mineral N between vegetation types. If anything there appears to be an enhancement in NH_4^+ concentration in the grassland samples relative to the forest samples. Further inspection, however, reveals enhanced NO_3^- per kg dry mass of soil relative to the total anionic suite in the forest samples, while NH_4^+ status appears to be essentially the same between vegetation types. Similarly, KCl-extractable NO_3^- is enhanced in the forest samples relative to the grassland samples, significant at the 94% confidence level. Extractable NH_4^+ is also enhanced in the forest samples. The difference, however, is not significant due to the large variance within vegetation groups.

In general, the inclusion of the organic forest litter horizons in the sampling has proved to be instructive. Previous studies investigating differences in chemical soil properties resulting from afforestation in the eastern escarpment concentrated on the mineral topsoil and excluded the forest floor horizons in the sampling strategy (Nowicki, 1997; Sugarman, 1999). Organic matter content has been a crucial variable influencing total N, CEC_e, EC and Al chemistry in the forest samples. Further, there appears to be a distinction in organic matter quality between vegetation types that warrants additional research.

Another point of interest that arises from the general chemical assessment of the samples is the possible existence and role of AEC. The decrease in ΔpH with decreasing pH coupled with the enhanced NO_3^- in the KCl extracts relative to the saturated paste extracts suggest the possibility of protonated (metal complexed) organic matter exhibiting a pH dependent anion attenuation capacity. In order to assess the possibility of NO_3^- sorption and to assess differences in N transformations contributing to differences in N status between vegetation types, a series of laboratory experiments was conducted. The results of this experimental study are discussed in the following chapter.

Chapter 3. Laboratory experiments: differences in N mobility resulting from vegetation

3.1 Introduction

In the previous chapter an enhanced N status in the forest samples relative to the grassland samples was identified, specifically in terms of total N, soil solution NO_3^- and KCl-extractable NO_3^-. In order to understand the differences in soil N status between vegetation types it is necessary to examine N transformations influencing N status. Interesting observations from the previous chapter have also provoked questions regarding NO_3^- sorption which could influence N mobility in the soils. The laboratory experiments reported in this chapter were designed to assess N mobility in terms of NO_3^- sorption, soil : extract ratio dilution effects, as well as aerobic and anaerobic mineralisation rates.

3.2 Methods

3.2.1 Experimental design

A brief description of the laboratory experiments follows. Further details describing the experimental methodology and analytical techniques appear in Appendix B.

A sorption experiment was conducted to investigate the NO_3^- sorption capacity of the soil samples. Four samples (ggs2, gfs3, kgs4 and kfs3) characterised by high acidity and low pH were chosen for the experiment. Five treatments were used: 0.0, 3.4, 6.2, 10.7, and 14.2 mmol KNO_3/kg. Five grams of sample was equilibrated with 25 ml solution for 30 minutes on a shaker. The supernatant of the soil-solution mixture was filtered through a 0.2 μm filter. All samples, except for the 0 treatment, were then diluted 10 times. Samples were analysed for major anions with ion chromatography as described in Chapter 2.

A dilution experiment was conducted in order to assess the effect of varying soil : extract ratios on the amount of extractable NO_3^-. Deionized water was used for extraction and soil : water ratios of 1:1, 1:2, 1:5, and 1:10 were employed. The experiment was conducted with samples ggs2 (air-dried) and gfs3 (field-moist).

Two N mineralisation experiments were conducted: an aerobic and anaerobic incubation. Field moist sample that had been refrigerated was used for both mineralisation experiments. The aerobic experiment was designed after Schmidt and Belser (1982). An oven-dried equivalent mass of 25 g of sample was treated with 250 µmol of NH_4^+ in the form of $(NH_4)_2SO_4$ solution. Water was added until field capacity was reached (field capacity was taken to be half of saturation which had been estimated by making saturated pastes - Chapter 2). The experiment was run in duplicate and included control samples which had not been treated with NH_4^+. The duplicate samples and controls were incubated at ~25-30°C for 28 days. The NO_3^- at time 0, 14 and 28 days was extracted with 2 M KCl, filtered through a Whatman #1 qualitative filter and analysed using the copperized cadmium method (Stock, 1983).

The anaerobic experiment was designed after Keeney (1982). An oven-dried equivalent mass of 5 g of sample was waterlogged with 15 ml of deionized H_2O. The samples were sealed and incubated at 40°C for 7 days. The experiment was run in duplicate and relative to controls that were kept frozen at 0°C over the same time period. The water-logged samples were extracted with 15 ml of 2 M KCl after 7 days, filtered through a Whatman #1 qualitative filter and analysed for NH_4^+ using the indo-phenol blue method (Keeney and Nelson, 1982).

3.2.2 Statistical interpretation

For the same reasons listed in Chapter 2, non-parametric statistics were used for interpretation purposes. Correlations between variables were evaluated with Spearman's rank correlation statistic and significance of difference due to vegetation was evaluated by Mann-Whitney U test (Chapter 2). The cases used to evaluate correlations and significance of difference was limited to the sub-set of thirteen samples identified in Chapter 2 for which vegetation is a legitimate grouping variable.

Emphasis was placed on the reproducibility of the experimental results which was evaluated with the average of repeats, standard deviation (SD) and relative standard deviation (RSD) defined as the SD divided by the average multiplied by 100 to give a percentage. In general, experiments with a median RSD greater than 25% were considered non-reproducible and thus inconclusive.

3.3 Results and discussion

The results of the experiments are listed in Table 3.1.

3.3.1 Nitrate sorption

In the previous chapter NO_3^- concentrations in saturated paste extracts and in 2 M KCl extracts were compared. The comparison revealed a significant enhancement in the KCl extracts relative to the saturated paste extracts. A possible explanation is that an anion exchange capacity exists in the samples, resulting in a mass action effect when soil samples are extracted with KCl where the Cl^- ions replace anions in general and NO_3^- specifically on exchange sites. Lending weight to this hypothesis was an apparent decrease in ΔpH with decreasing pH resulting, perhaps, from converging magnitudes of AEC and CEC. Furthermore, the difference in NO_3^- concentration between the vegetation types was more significant in the KCl extracts ($p = 0.06$) than in the saturated paste extracts ($p = 0.12$), where both are expressed on a per dry mass basis, perhaps suggesting a larger AEC in the forest samples. Similarly, there was a significantly lower ΔpH in the forest samples (median ΔpH = 0.69) relative to the grassland samples (median ΔpH = 0.93; $p = 0.018$; Figure 3.1), again suggesting the possibility of an enhanced AEC in the forest samples relative to the grassland samples.

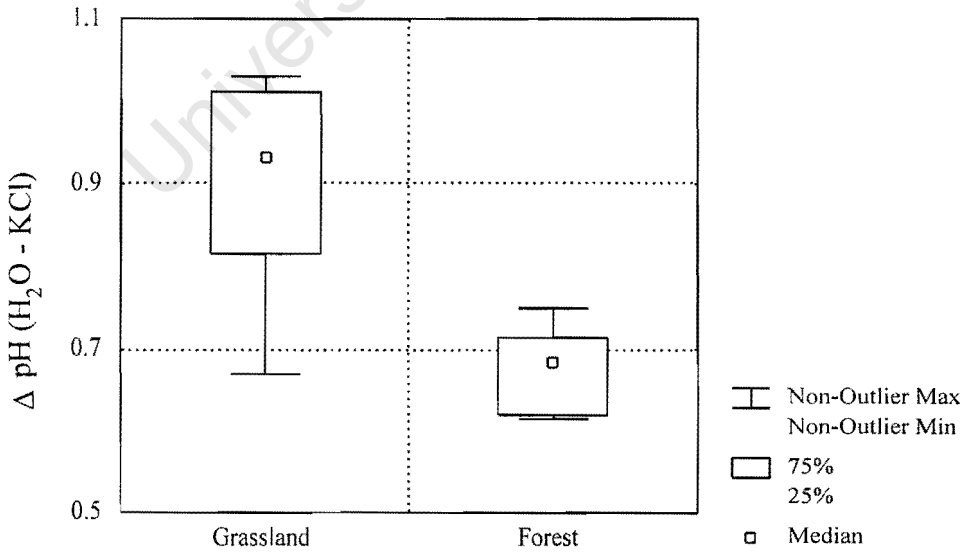


Figure 3.1 Box and whisker plot illustrating the significantly lower Δ pH in the forest samples relative to the grassland samples ($p = 0.018$).

Table 3.1 Results of laboratory experiments investigating differences in N mobility resulting from vegetation for forest and grassland soil samples from the Graskop and Kaapsehoop areas of the eastern escarpment, South Africa.

Location	Graskop										Kaapsehoop									
Vegetation	Grassland					Forest					Grassland					Forest				
Sample ID	ggs1	ggs2	ggs3	ggs4	ggs5	gfs1	gfs2	gfs3	gfs4	gfs5	kgs1	kgs2	kgs3	kgs4	kgs5	kfs1	kfs2	kfs3	kfs4	kfs5
Sorption treatments KNO_3 added mmol kg^{-1}; results reported as NO_3 sorbed (NO_3 added – NO_3 in equilibrium solution) mmol kg^{-1}																				
0.0	0.00					-0.10					-0.89					-0.11				
3.4	0.19					0.78					0.22					0.77				
6.2	-1.12					-0.08					-0.67					0.27				
10.7	-0.53					1.41					1.95					0.81				
14.2	-1.99					0.71					0.39					-0.83				
Dilution soil: extract (m/v) - NO_3 (mmol kg^{-1})																				
1:1	0.06					0.12														
1:2	0.03					0.10														
1:5	0.01					0.18														
1:10	0.02					0.09														

Table 3.1 Results of laboratory experiments (continued)

Sample ID	ggs1	ggs2	ggs3	ggs4	ggs5	gfs1	gfs2	gfs3	gfs4	gfs5	kgs1	kgs2	kgs3	kgs4	kgs5	kfs1	kfs2	kfs3	kfs4	kfs5
Aerobic mineralisation experiment - NO_3 (mmol kg^{-1})																				
Day 0	0.19	0.17	0.15	0.15	0.14	0.27	0.28	0.25	0.41	0.14	0.08	0.14	0.72	0.41	0.54	0.53	0.18	0.16	0.34	0.14
RSD (%)	4.1	3.5	**44.7**	10.8	20.6	0.6	17.0	**34.3**	18.0	19.4	**67.7**	2.1	0.9	23.6	8.9	20.0	23.5	11.4	**26.4**	**46.6**
Day 14 control	0.00	0.00	0.00	0.00	0.00	0.55	0.27	0.00	0.27	0.21	0.50	0.25	0.55	5.13	2.34	1.53	0.94	0.00	1.21	0.89
Day 14	0.24	0.75	0.52	0.42	1.14	0.34	1.75	0.69	0.28	0.72	0.58	0.08	0.40	1.23	2.79	1.25	0.60	0.07	0.80	0.52
RSD (%)	**101.3**	7.6	**46.3**	**113.8**	**112.6**	**83.4**	**40.9**	**48.1**	**110.4**	**97.4**	**75.6**	**589.2**	**79.5**	**68.4**	**48.1**	18.6	**147.7**	**244.2**	20.2	**49.4**
Day 28 control	0.07	0.33	0.10	0.39	0.00	0.99	1.17	0.89	1.78	1.16	0.25	0.07	1.11	3.19	1.74	2.77	2.96	0.20	3.02	3.07
Day 28	0.17	0.50	0.54	0.94	3.15	0.97	4.59	0.66	1.85	0.63	1.38	0.48	2.25	1.76	4.29	3.86	2.50	0.21	2.12	3.13
RSD (%)	18.5	16.1	1.7	**30.6**	2.9	**25.4**	**55.9**	**50.0**	na	**85.7**	6.9	**61.6**	**65.1**	20.9	12.2	13.7	1.7	na	20.8	**58.9**
Anaerobic mineralisation experiment																				
NH_4 mmol kg $soil^{-1}$ day^{-1}	0.36	0.15	0.20	0.04	0.16	0.36	0.23	0.26	0.60	0.22	0.23	0.43	0.44	0.05	0.24	0.34	0.52	0.61	0.55	0.36
RSD (%)	**48.5**	16.3	12.4	17.1	19.6	na	na	2.0	11.4	**28.3**	17.8	11.8	0.2	**34.1**	3.8	19.3	12.1	24.3	7.9	8.4
NH_4 mmol $kgOC^{-1}$ day^{-1}	3.37	5.25	7.00	4.43	4.67	3.91	2.70	5.84	6.11	6.45	7.01	8.11	4.52	3.53	3.66	8.65	8.35	7.40	8.39	8.13

The sorption experiment consisted of equilibrating four soil samples with various amounts of KNO_3 (0.0, 3.4, 6.2, 10.7, 14.2 mmol kg^{-1}) and measuring the NO_3^- concentration in the equilibrium solution. The results of the experiment are listed in Table 3.1. The results suggest a consistent pattern of adsorption with the lowest addition of KNO_3 (3.4 mmol kg^{-1}) for all samples. Despite this pattern, it was impossible to construct meaningful sorption isotherms due to the sporadic results obtained for the treatments with higher concentrations, and in this respect, the experiment was considered inconclusive. It is likely that the KNO_3 treatment sequence was too concentrated for the hypothetical AEC of the soils explaining the consistent trend of sorption between the first two treatments and the sporadic results for the higher concentrations. It is possible that the AEC became NO_3^- saturated at the lower concentrations and the higher concentrations, by nature of the magnitude, are characterised by analytical error compounded by the dilution factor employed to obtain resolution within the calibration range of the analysis. Another inherent flaw in the experiment was the varying ionic strength between treatments. Surface charge generally increases with ionic strength (Rowell, 1994), and perhaps this inconsistency between treatments contributed to the nonsensical pattern of results. Although it might have been useful to repeat the experiment with lower NO_3^- concentrations and a background electrolyte solution, this would only be instructive if there was some measure of specificity associated with NO_3^- adsorption. Since NO_3^- adsorption is essentially non-specific (McBride, 1994), the only means of clarifying whether the results in Table 3.1 do reflect some measurable NO_3^- adsorption at low levels of NO_3^- addition would be to employ a rigorous method for AEC determination in variable charge soils such as the compulsive exchange method of Gillman (1979).

3.3.2 Dilution

The possibility that soil : extract ratio influences the amount of extractable NO_3^- was also suggested in Chapter 2. This consideration arose again from the enhanced NO_3^- concentrations in the KCl extracts relative to the saturated paste extracts. Since the soil : extract ratios were not the same for both techniques (1:10 for the KCl extracts compared to ~ 1:0.5 for the saturated paste extracts), it was considered that a larger extraction volume might result in a larger amount of extractable NO_3^- per kg of soil.

This is predictable in terms of Fick's first law of diffusion, which states that:

$$J = -D\,\partial C/\partial x$$

Where J = flux: amount of constituent moving across an area per unit time, D is the diffusion constant and $\partial C/\partial x$ = concentration gradient. Simply put, Fick's first law predicts that a larger concentration gradient will result in a larger flux (Kutílek and Nielsen, 1994). A larger extraction volume coincides with a larger concentration gradient. It is expected, therefore, that the larger volume (KCl extract) will extract more solute for a given mass of soil than the smaller volume (saturated paste extract). Consequently, in order to assess the effect of varying soil : extract ratios on extractability of NO_3^-, the dilution experiment was conducted, which consisted simply varying the soil : solution ratio of aqueous extraction.

The result of the dilution experiment with respect to NO_3^- are presented in Table 3.1 and the results for all cations and anions are presented in Appendix C. The results suggest that the amount of NO_3^- extracted per kg of soil is not dependent on the soil : extract ratio. If anything, it appears that the amount of NO_3^- extracted decreases with decreasing soil : extract ratio. This is not, however, significant. Caution needs to be employed when interpreting the results, however, due to inconsistent and spurious results of the complete cation and anion suite. Anomalous concentrations of SO_4^{2-}, Cl^-, Na^+ and NH_4^+ (Appendix C) suggested the possibility of contamination. Consequently, although the results point to a negligible effect of soil : extract ratio with respect to NO_3^- extractability, the experiment should be regarded as being inconclusive.

3.3.3 Aerobic mineralisation

An aerobic mineralisation experiment was conducted with an NH_4^+-N amendment in order to determine the nitrifying potential of the soils. In untreated soil the rate of NO_3^- formation is generally limited by the rate at which NH_4^+ is formed from ammonification of organic N. The addition of NH_4^+-N ensures that the soil will be non-limiting with respect to nitrifiable substrate and the nitrifying population is, therefore expected to increase until limited by some other factor or combination of factors. The experiment is limited in its application due to the absence of vegetation and of temperature and moisture fluctuations. It is, however, useful as a means of comparing different soils with different properties (Schmidt and Belser, 1982).

The results of the aerobic mineralisation experiment are listed in Table 3.1. Figures 3.2, 3.3 and 3.4 show the average and duplicate results of day 0, 14 and 28, respectively. Day 0 and 28 results are reproducible on average within 20%, whereas day 14 results are not reproducible and no attempt will be made to interpret them. A contributing factor to the non-reproducibility of the experiment was the lack of standardisation between duplicates particularly with respect to airflow and moisture content, both of which influence the redox potential of the system. It is possible that anaerobic microsites were induced due to the inadvertent variation in treatment of soil samples. Due to the inherent problems in the reproducibility of the experiment, only day 0 and day 28 results will be interpreted in the broadest of terms.

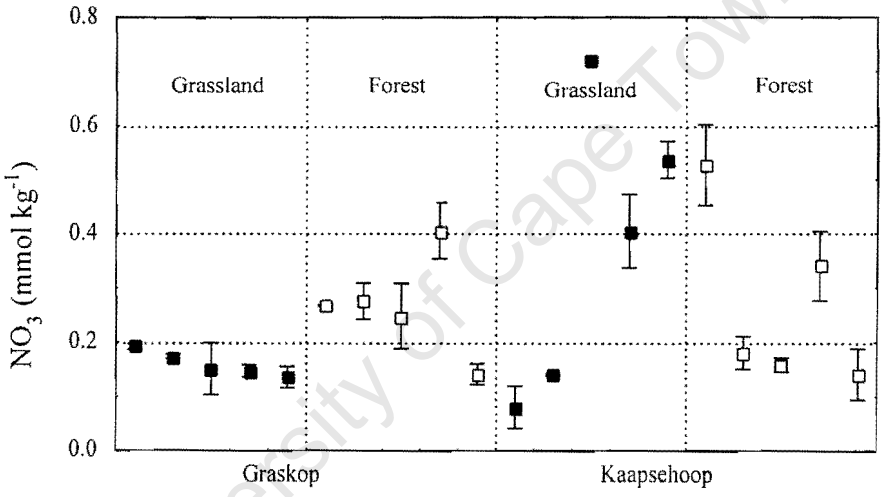


Figure 3.2 Results of day 0 aerobic mineralisation experiment including the average, maximum and minimum repeats. The range of RSD is 0.6 - 67.7% (median = 18.7%) for all samples and 0.6 - 67.7% (median = 20.0%) for the sub-set of thirteen samples.

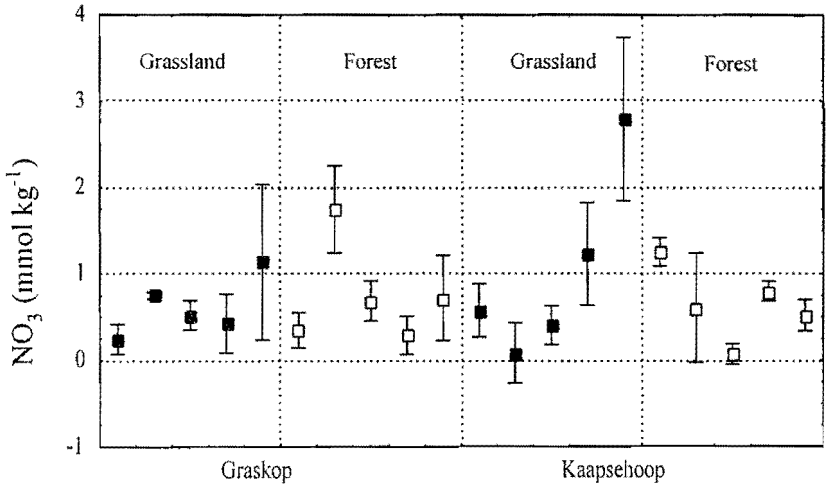


Figure 3.3 Results of day 14 aerobic mineralisation experiment for all samples including the average, maximum and minimum repeats. The range of RSD is 8-589% (median = 78%) for all samples and 8-148% (median = 79%) for the sub-set of thirteen samples.

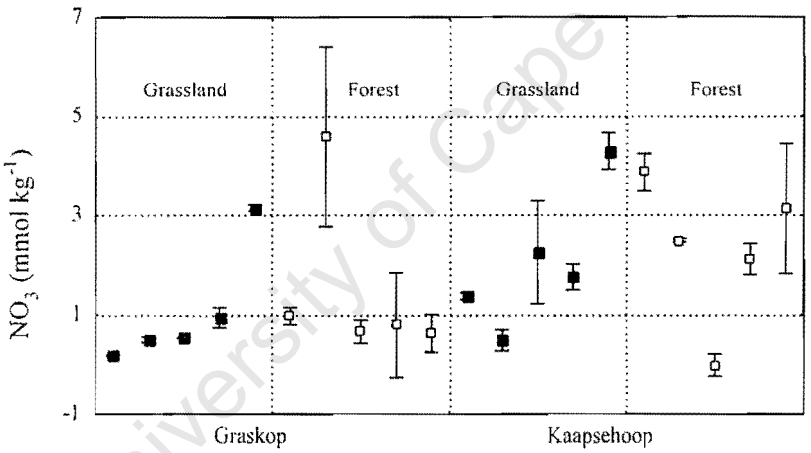


Figure 3.4 Results of day 28 aerobic mineralisation experiment including the average, and maximum and minimum. The range of RSD is 1.7-85.7% (median 20.9%) for all samples and 1.7-85.7% (median 18.5%) for the sub-set of thirteen samples.

The day 0 results reveal a significant enhancement in KCl extractable NO_3^- in the forest samples relative to the grassland samples ($p = 0.06$; Figure 2.3). The day 28 results, contrarily, reveal no significant difference between vegetation types ($p = 0.25$). Similarly, however, to the day 0 results, the day 28 control (samples not treated with $(NH_4)_2SO_4$) reveal a significant NO_3^- mineralisation enhancement in the forest samples relative to the grassland samples ($p = 0.05$; Figure 3.5). Figures 3.6 and 3.7 show the case profile trends between day 0 and day 28 control and treatment, respectively. In general, the slope for the forest trends between day 0 and day 28 control are steeper than the trends for the grassland samples (Figure

3.6). This is not the case, however, for the day 0 and day 28 treatment trends, which are characterised by overlapping slopes for both vegetation types (Figure 3.7).

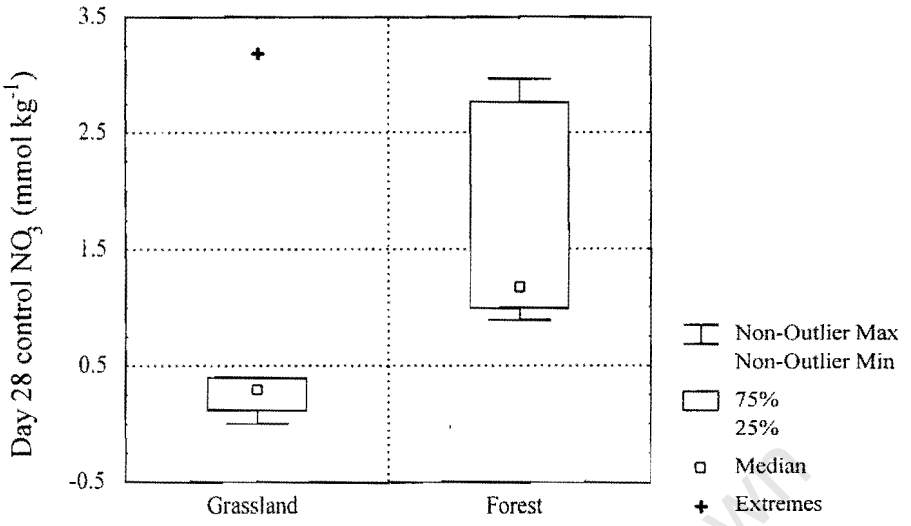


Figure 3.5 Box and whisker plot illustrating the difference in aerobic NO_3 produced after 28 days (control) between vegetation types ($p = 0.05$).

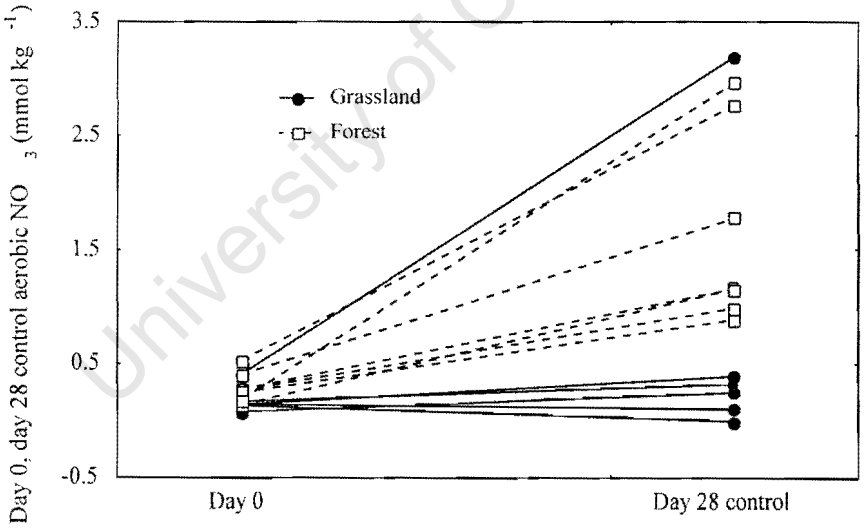


Figure 3.6 Case profile of the trend of aerobic mineralisation, day 0 and 28, of control treatment.

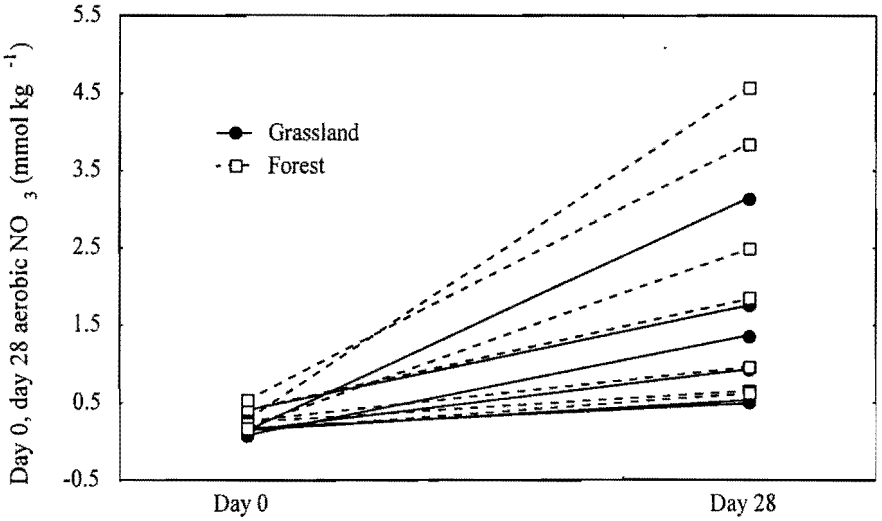


Figure 3.7 Case profile of the trend of aerobic mineralisation, day 0 and 28, of $(NH_4)_2SO_4$ treatment.

Calculating rates of net nitrification (mmol NO_3 kg $soil^{-1}$ day^{-1}) for day 28 (control) relative to day 0 reveals an enhancement in the forest samples compared to the grassland samples, significant at the 95% confidence level (Figure 3.8). Table 3.2 lists correlation coefficients for rate of net nitrification and soil properties that may contribute to or may be influenced by the enhanced rate in the forest samples relative to the grassland samples. The enhanced rate of net nitrification (control) in the forest samples relative to the grassland samples is correlated neither with organic matter status nor with total N status. There is, however, a slight negative correlation between rate of net nitrification and C/N ratio (r_s = -0.42, p = 0.16) and the significance of the correlation is improved for the forest samples alone (r_s = -0.68, p = 0.09, n = 7). The inverse relationship between rate of nitrification and C/N is well-documented in forest soils, specifically the C/N in the forest floor (e.g. Van Miegroet *et al.*, 1989; Emmett *et al.*, 1998; Gundersen *et al.*, 1998). Emmett *et al.* (1998) report that a decrease in C/N ratio of the forest floor necessarily pre-empts the stimulation of nitrification. There also appears to be a slight inhibiting effect of decreasing soil pH on rate of net nitrification, although not highly significant (r_s = -0.48, p = 0.09). There does not appear to be a soil chemical property that has been measured that can fully account for the enhanced rate of net nitrification in the forest samples relative to the grassland samples. It is possible that there are differences in soil microbiological and/or biochemical properties, particularly with respect to the nitrifying communities, between the vegetation types contributing to the enhanced rate of net nitrification in the forest samples. The enhanced rate in the forest samples does, however, seem to account for the enhanced soil solution and extractable NO_3^-

status in the forest samples relative to the grassland samples. Figure 3.9 shows the relationship between extractable NO_3^- and rate of net nitrification.

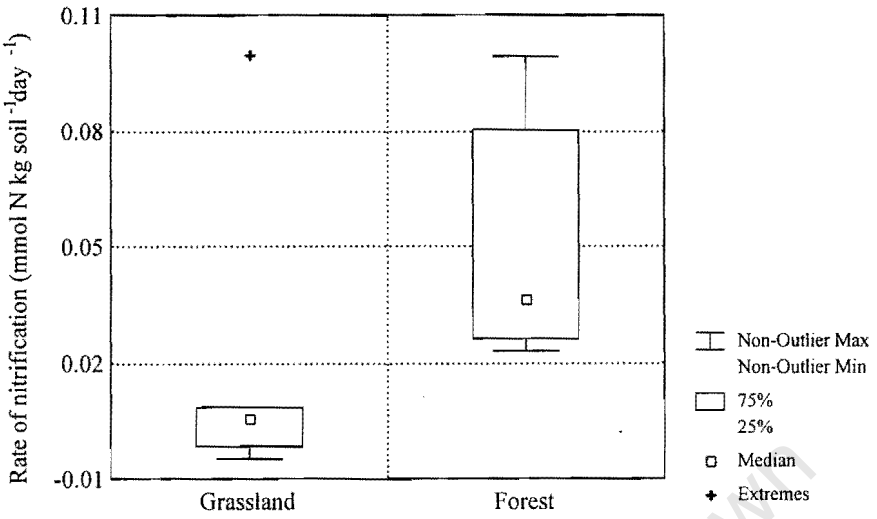


Figure 3.8 Box and whisker plot illustrating difference in rate of net nitrification for untreated control between vegetation types ($p = 0.05$).

Table 3.2 Spearman's correlation coefficient for rate of net nitrification (control) and selected variables.

	All samples (n = 13)
Organic carbon (%)	0.31
Total nitrogen (%)	0.27
C/N[1]	-0.42
pH (H_2O)	-0.48
NO_3/I[2]	0.68**
Extractable NO_3 (mmol kg^{-1})	0.69**

*, **, *** – Significant at the 95%, 99% and 99.9% confidence level, respectively
[1] correlation between rate of net nitrification and C/N for forest samples: $r_s = -0.68$, $p = 0.09$
[2] where NO_3/I is soil solution NO_3 (mmol kg^{-1})/ ionic strength (mmol kg^{-1})

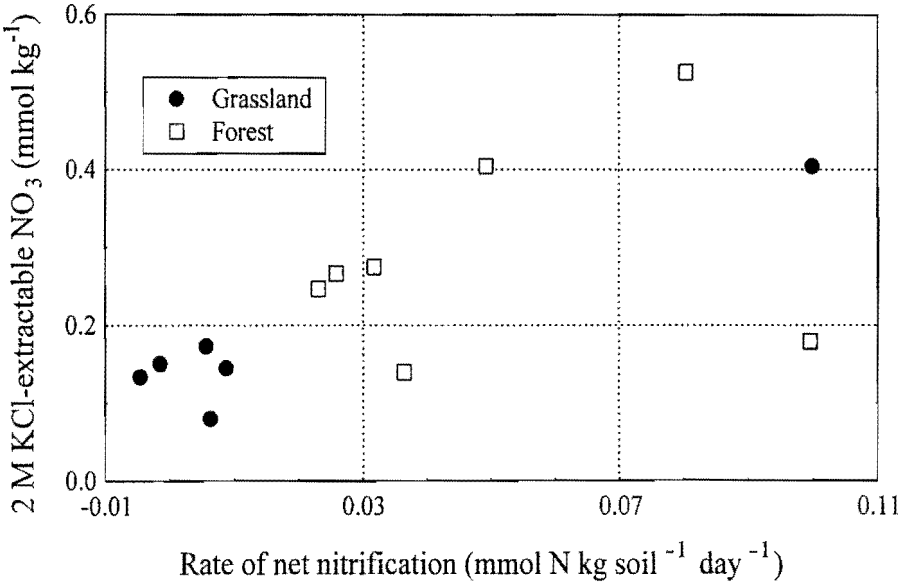


Figure 3.9 The relationship between extractable NO_3^- and rate of net nitrification ($r_s = 0.69$, $p = 0.01$).

Calculating rate of net nitrification between day 28 (treated) and day 0 reveals no significant difference in rate between the grassland and forest samples ($p = 0.32$; Figure 3.10). Furthermore, calculating the difference between nitrification rates of treatment and control (Δ nitrification) reveals a median value of 0.017 in the grassland samples compared to -0.001 mmol NO_3^- kg $soil^{-1}day^{-1}$ in the forest samples ($p = 0.39$). Although the difference in Δ nitrification between vegetation types is not significant, it suggests that adding $(NH_4)_2SO_4$ actually impedes the net nitrification rate in forest samples while enhancing it, albeit slightly, in the grassland samples.

The retarding of net nitrification in forest samples might arise from the acidifying effect of $(NH_4)_2SO_4$ (Tisdale, *et al.*, 1985) which may inhibit nitrification rate in the initially more acidic forest soils more than it does in the less acidic grassland soils. This is substantiated by the significant positive correlation between Δ nitrification and pH (H_2O) ($r_s = 0.77$, $p = 0.002$; Figure 3.11). This trend suggests that with increasing pH, the addition of $(NH_4)_2SO_4$ enhances net nitrification rate and, conversely, with decreasing pH, $(NH_4)_2SO_4$ has a negligible to inhibiting effect on the rate of net nitrification, suggesting in turn that the difference between vegetation types with respect to Δ nitrification stems from an overall difference in acidity status. Another possible interpretation of the data is that nitrification, rather than ammonification from organic matter, is the rate limiting process in the formation of NO_3^- in the forest soils, thus accounting for the negligible to inhibiting effect of $(NH_4)_2SO_4$

amendment. It is also possible that the addition of the amendment induced denitrification in the forest samples, such that Δ nitrification approached zero.

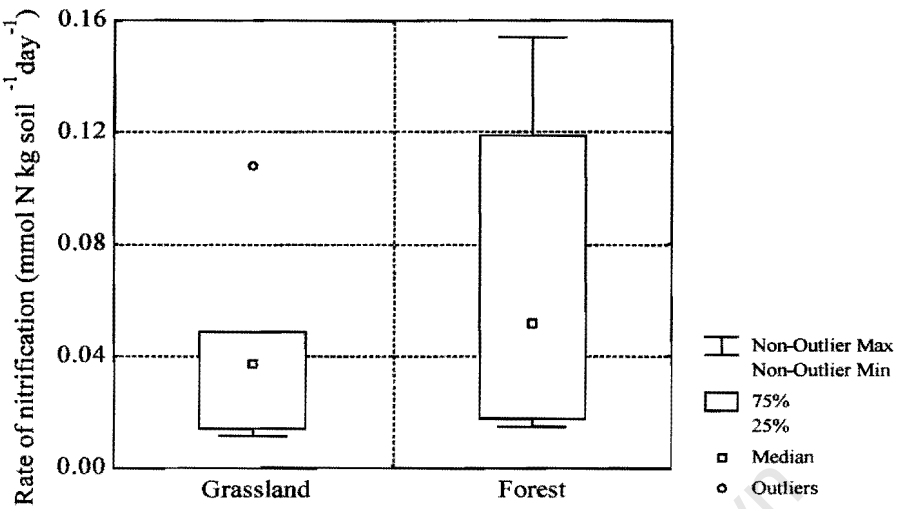


Figure 3.10 Box and whisker plot illustrating difference in rate of net nitrification for $(NH_4)_2SO_4$ treatment experiment between vegetation types ($p = 0.32$).

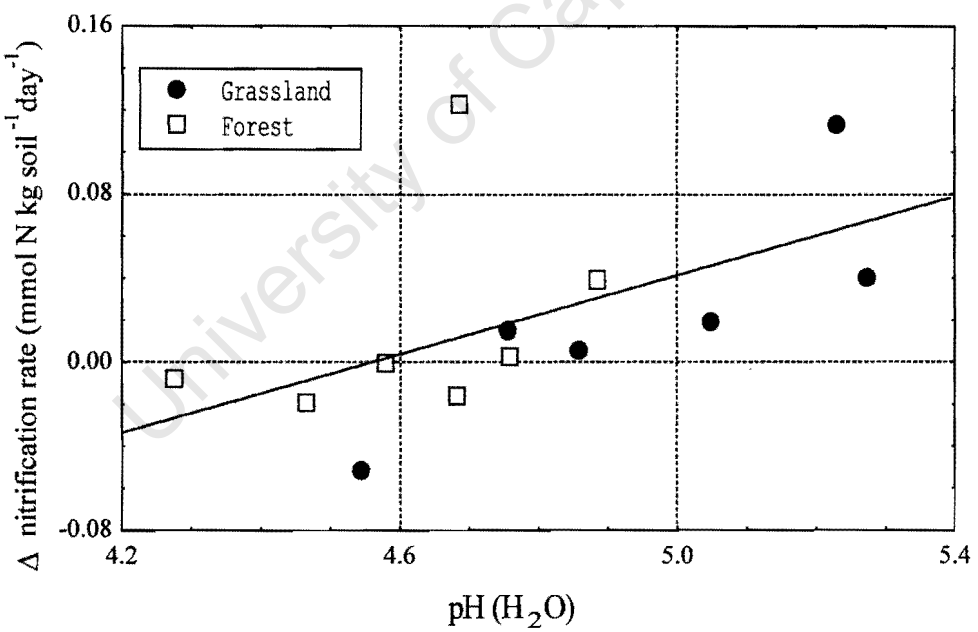


Figure 3.11 The correlation between Δ nitrification rate (the impact of $(NH_4)_2SO_4$ amendment) and pH (H_2O) ($r_s = 0.77$, $p = 0.002$).

3.3.4 Anaerobic mineralisation

An anaerobic mineralisation experiment was conducted under waterlogged conditions in sealed vessels. In the waterlogged incubation, biological activity is expected to maintain

anaerobic conditions, eliminating nitrification-denitrification reactions at the soil-water interface. Since ammonification is expected to be rate limiting in NO_3^- formation, the anaerobic method provides a suitable index of N availability. Advantages of the anaerobic method compared to the aerobic method include standardised conditions with respect to water content and the use of higher temperatures (40°C) (which ensures rapid mineralisation) since optimum temperature is not as crucial as it would be in the case of nitrification (Keeney, 1982). As with the aerobic incubation, however, the method is limited in application due to the absence of vegetation and of temperature and moisture fluctuations, but it does provide a means of useful comparison between soils.

The results of the anaerobic incubation are presented in Table 3.1 and the average and range of duplicates are shown in Figure 3.12.

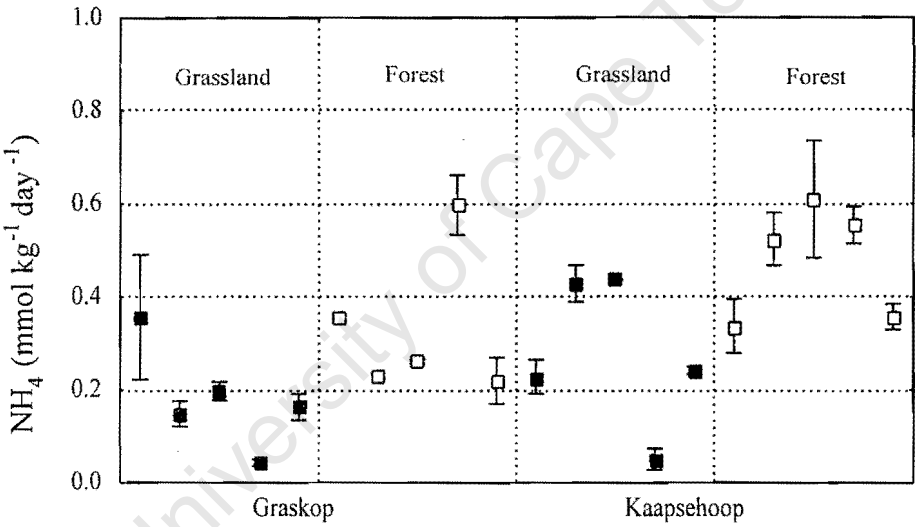


Figure 3.12 Results of anaerobic mineralisation experiment for all samples including the average, maximum and minimum repeats. The range of RSD is 0.2-48.5% (median 14.4%) for all samples and 11.4-34.2% (median 17.1 %) for the sub-set of thirteen samples.

The impact of afforestation on N mineralisation will be assessed by considering the results of the anaerobic mineralisation experiment for the sub-set of 13 samples for which vegetation is a valid clustering variable (Chapter 2). Figure 3.13 shows a box and whisker plot of the NH_4^+ produced per kg of soil per day in the anaerobic mineralisation experiment. The range of NH_4^+ is 0.04-0.23 with a median of 0.16 mmol kg $soil^{-1}day^{-1}$ for the grassland samples and a range of 0.22-0.60 with a median of 0.34 mmol kg $soil^{-1}day^{-1}$ for the forest samples, a difference significant at the 99% confidence level. The enhancement in the forest samples is

attributed singularly to the higher total N content, stemming from the enhancement of organic matter content (Chapter 2). This is substantiated by the significant correlation between NH_4^+ produced and total N (Figure 3.14). Expressing the rate of mineralisation relative to organic C content in fact eliminates the difference in rates between vegetation types (Figure 3.15). It follows that, although the total amount of mineralisable N per mass is greater in the forest samples, the fraction of total N that is mineralisable is virtually the same between vegetation types.

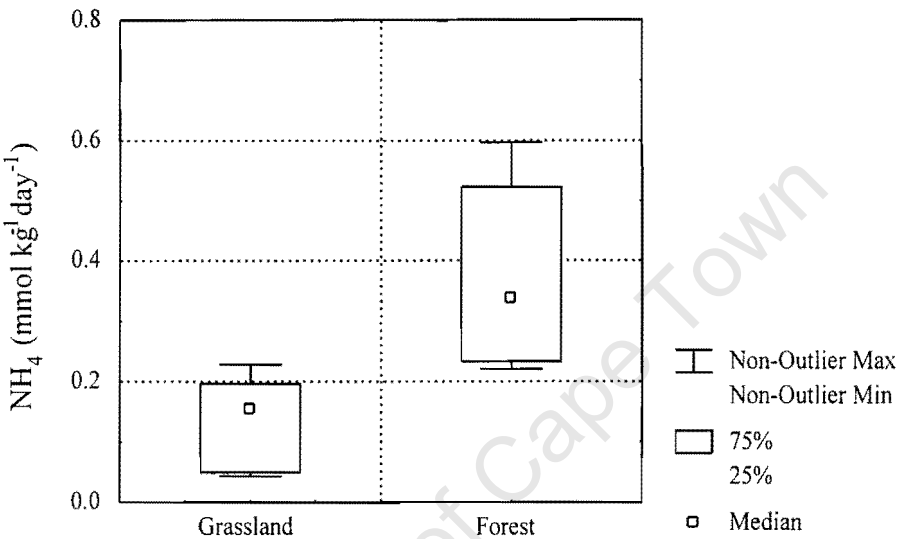


Figure 3.13 Box and whisker plot illustrating difference in anaerobic mineralisation experiment between vegetation types ($p = 0.004$).

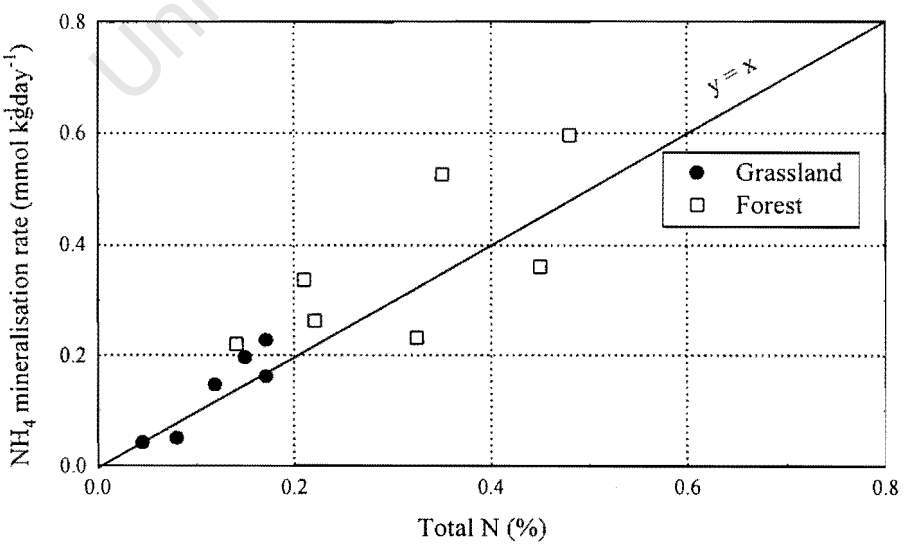


Figure 3.14 The significant correlation between NH_4^+ (mmol kg $soil^{-1}day^{-1}$) and total N ($r_s = 0.94$, $p < 0.001$).

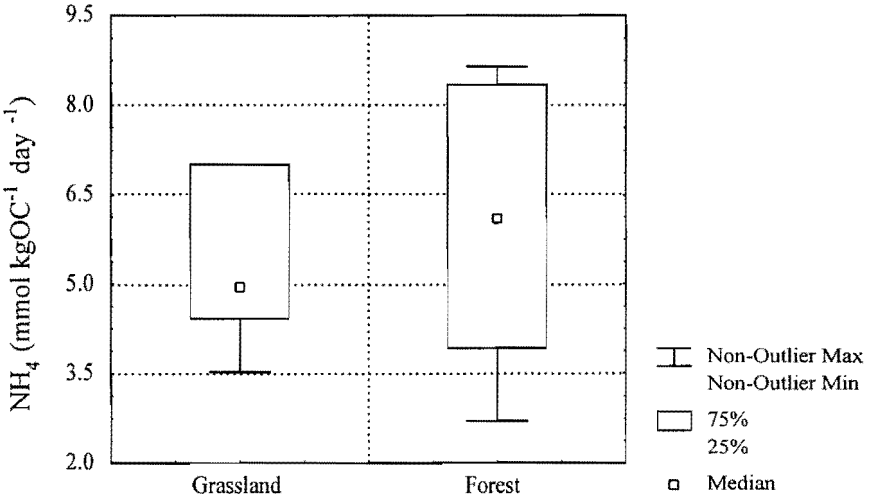


Figure 3.15 Box and whisker plot comparing results of anaerobic experiment - NH_4 produced relative to organic carbon content - between vegetation types ($p = 0.57$).

In order to assess possible variables influencing mineralisation rate, Spearman's correlation test was employed. Tables 3.3 lists the results of the Spearman's correlation test (r_s) between mineralised NH_4^+ (mmol kg $soil^{-1}day^{-1}$) and selected variables for all samples, grassland samples and forest samples. It is clear from the results of the tests that total N is most strongly related to mineralised NH_4^+ for all samples, which is expected (Figure 3.14). The other variables for which all samples exhibit a correlation are attributed to the correlation between mineralised NH_4^+ and organic C. As discussed in Chapter 2, total N, CEC_e, and acidity are all significantly correlated with organic C content and it has been theorised that the correlations are causal in all three cases.

Similarly to Table 3.3, Table 3.4 lists the results of the Spearman's correlation test (r_s) between mineralised NH_4^+ (mmol $kgOC^{-1}day^{-1}$) and selected variables for all samples, grassland samples and forest samples. The results of the correlation tests are ambiguous at best; there is not a single variable that is significantly correlated to the rate of mineralisation for all samples. There is a slight negative correlation between the mineralisation rate and C/N ($r_s = -0.52$, $p = 0.07$) and a slight positive correlation with soil solution NH_4^+ concentration ($r_s = 0.52$, $p = 0.07$) for all samples. The rate of mineralisation in the forest samples, however, is negatively correlated with C/N, CEC_e and acidity. The relationship between mineralisation rate and C/N is shown in Figure 3.15.

Table 3.3 Spearman's correlation coefficient (r_s) for NH_4 produced in the anaerobic mineralisation experiment (mmol kg $soil^{-1}day^{-1}$). and selected variables for all samples, grassland samples and forest samples.

	All samples n = 13	**Grassland n = 6**	**Forest n = 7**
Organic C (%)	0.90***	0.71	0.68
Total N (%)	0.94***	0.90**	0.82**
C/N	-0.29	0.09	-0.71
CEC_e ($mmol_c kg^{-1}$)	0.86***	0.77	0.39
Acidity ($mmol_c kg^{-1}$)	0.80***	0.54	0.18
NO_3 ($mmol\ kg^{-1}$)	0.59*	0.37	0.57
NH_4 ($mmol\ kg^{-1}$)	0.45	0.31	0.93**
Extractable NO_3 ($mmol\ kg^{-1}$)	0.47	-0.54	0.36
Extractable NH_4 ($mmol\ kg^{-1}$)	0.23	-0.43	0.04
EC (μScm^{-1})	-0.45	0.26	0.86**

*, **, *** – Significant at the 95%, 99% and 99.9% confidence level, respectively

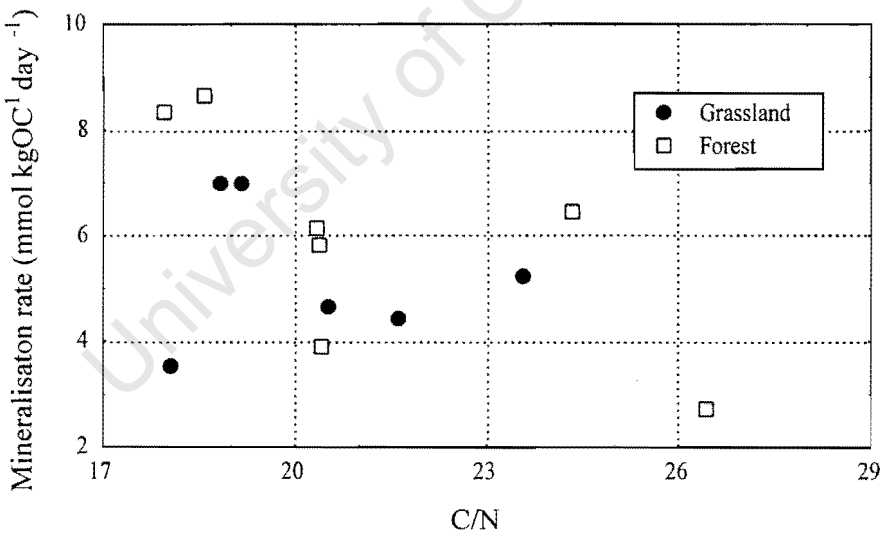


Figure 3.16 The relationship between rate of mineralisation (calculated from anaerobic incubation) and C/N (r_s = -0.52, p = 0.07).

Table 3.4 Spearman's correlation coefficient for NH_4 produced in the anaerobic mineralisation experiment (mmol $kgOC^{-1}day^{-1}$) and selected variables for all samples, grassland samples and forest samples.

	All samples n = 13	**Grassland n = 6**	**Forest n = 7**
Organic C (%)	0.04	0.54	-0.54
Total N (%)	0.15	0.70	-0.36
C/N	-0.52	0.09	-0.75*
CEC_e ($mmol_c kg^{-1}$)	0.01	0.49	-.079*
Acidity ($mmol_c kg^{-1}$)	-0.07	0.20	-0.86**
NO_3 ($mmol\ kg^{-1}$)	-0.07	-0.03	0.00
NH_4 ($mmol\ kg^{-1}$)	0.52	0.66	0.43
Extractable NO_3 ($mmol\ kg^{-1}$)	-0.13	-0.54	0.00
Extractable NH_4 ($mmol\ kg^{-1}$)	-0.48	-0.77	-0.46
EC (µS/cm)	0.14	0.26	0.50

*, **, *** – Significant at the 95%, 99% and 99.9% confidence level, respectively

3.4 General discussion and conclusions

The sorption experiment, designed to examine the possibility of NO_3^- sorption capacity specifically and of AEC generally, was inconclusive, despite the consistent positive NO_3^- sorption at the lowest level of KNO_3 addition. Further studies are needed to investigate the NO_3^- sorption capacity of the soils. Future studies should use a narrower range of NO_3^- concentrations and should attempt to standardise ionic strength between treatments. A better alternative might be to directly determine AEC using a method which accommodates the ionic strength effect in variable charge soils. It would also be interesting to investigate NO_3^- sorption capacity and AEC with soil depth, since sub-soil horizons may contain more positively charged sites associated with a greater exposure of sesquioxide surfaces to the soil solution (White, 1997).

The results of the dilution experiment intended to test the effect of varying soil : extract ratio on extractable-NO_3^-, suggested that NO_3^- extractability is negligibly affected by soil: extract ratio. The results were also considered inconclusive, however, due to anomalous concentrations of other ions. The experiment should be repeated with a larger number of

samples and replication in order to assess the statistical significance of soil : extract ratio on ion concentration.

The results of the aerobic mineralisation experiment (day 0 and day 28) suggest that untreated, the forest samples are characterised by a higher rate of net nitrification than the grassland samples ($p = 0.05$). The enhancement in the forest samples is not related to enhanced organic matter status nor to total N status. There is a negative correlation between rate of net nitrification and C/N, although not highly significant ($r_s = -0.42$, $p = 0.16$). The enhanced rate of net nitrification in the forest samples does appear to account for the elevated soil solution and extractable NO_3^- in the forest samples relative to the grassland samples.

Treatment of soils with $(NH)_2SO_4$ results in a slightly inhibiting effect in the forest samples and a relative enhancement in the grassland samples. There is a significant correlation between the difference in treated and untreated nitrification rates (Δ nitrification) and pH ($r_s = 0.77$ and $p = 0.002$) suggesting that the acidifying effect of the amendment may retard the rate of net nitrification despite the non-limited availability of nitrifiable substrate. Other possible explanations for the lower Δ nitrification in the forest samples relative to the grassland samples might include forest NO_3^- formation not being limited by ammonification, or enhanced denitrification resulting from the amendment.

The results of the anaerobic mineralisation experiment suggest that a larger amount of N is mineralised per kg of soil in the forest samples relative to the grassland samples ($p = 0.004$). The enhancement in mineralisation in the forest samples is attributed singularly to the enhanced total N status, which, as discussed in Chapter 2, stems from the enhanced organic matter content due to the inclusion of the partially decomposed litter layer in the samples. Despite more N mineralisation in the forest samples, the proportion of total N that is mineralised is the same in soils of both vegetation types. In fact, there is no difference in mineralised N per kg of organic C between the forest and grassland samples.

Unlike the production of NO_3^-, the higher amount of NH_4^+ converted from organic N in the forest samples results from an overall higher organic matter content resulting from the larger biomass and accumulation of forest floor litter. The higher amount of NO_3^- produced in the forest samples, however, is not related to the higher organic matter content. There is a weak negative correlation between mineralised N per kg organic C and C/N ratio for all the samples and a more pronounced trend for the forest samples alone. Similarly, there is a weak negative

correlation between rate of net nitrification and C/N ratio (again, a stronger correlation exists for the forest samples). It appears as if C/N may be an important factor in predicting N mineralisation in the forest soils. Future studies should sample the forest floor and the mineral horizons separately to determine the role of each in N mineralisation.

Interpretation of the results of both mineralisation experiments was limited owing to the absence of vegetation and of temperature and moisture fluctuations. Gross mineralisation and nitrification rates may be very different from net rates observed using laboratory incubations. Internal cycling of NO_3^- by microbial biomass can be an important retention mechanism in coniferous forest soils and may vary with forest age (Emmett *et al.*, 1993). In order to truly assess N mineralisation rates and N fluxes in the study area, it would be necessary to conduct *in situ* incubations, perhaps using cation and anion exchange resin bags or tension lysimeters.

Chapter 4. General discussion and conclusions

The primary objectives of this study were, firstly, to examine and quantify the impact of pine plantations on soil N form and mobility in the eastern escarpment area of South Africa; secondly, to examine major processes associated with N status in an attempt at understanding differences between grassland and forest soils; and thirdly, to examine general soil chemical properties affected by afforestation and to assess relationships between these properties and soil N status.

The purpose of this chapter is to summarise the major findings of this study with particular emphasis on comparison with current literature and previous studies assessing the impacts of afforestation on soil chemical properties in the eastern escarpment of South Africa. Simultaneously, an attempt is made to relate the results of this thesis to the primary objectives set out at the onset of the study. Furthermore, results of a step-wise discriminant analysis are presented which pinpoint the soil chemical properties that discriminate between forest and grassland most efficiently. The chapter ends with a consideration of the practical implications of this study and recommendations for future work.

4.1 General characterisation of soils

General chemical and physical assessment of the soil samples has demonstrated a consistently highly leached, clay-poor, humus-rich, acidic nature of all the soils in the Graskop and Kaapsehoop study areas. The soil solution ionic composition is dominated by Na^+, K^+ and Cl^- and is characterised by low ionic strength with a median EC of 229 μScm^{-1}. Divalent cations (Ca^{2+} and Mg^{2+}) dominate the extractable base cation suite complementing the monovalent cation dominance in the soil solution. Extractable acidity is dominant relative to total extractable base cations and the samples are characterised by median acid saturation of 82%. The soils are characterised by generally low CEC_e, reflecting a highly leached status, which is most significantly correlated to organic C content suggesting that the majority of exchange sites are associated with humic substances. Aluminium speciation and solubility, although limited, tentatively suggests a tendency for organic matter buffering of Al particularly in the strongly acidic forest soils.

4.2 Impact of afforestation on soil chemical properties

In order to legitimately examine the impact of afforestation on soil chemical status, a series of cluster analyses was performed. The cluster analyses (Chapter 2) identified thirteen samples (six grassland and seven forest) for which vegetation is a legitimate grouping variable. These thirteen samples were then used alone to investigate the impact of afforestation on soil chemical properties.

4.2.1 Acidity status

The soil acidifying effect of pine plantations has been confirmed for the study area. The forest samples are characterised by lower pH, higher extractable acidity and acid saturation than the grassland samples. On average, the forest samples are characterised by almost five times more extractable acidity and a percentage acid saturation of six units higher than the grassland samples. The median acid saturation for the forest samples is 86%, which is the same as the average acid saturation Sugarman (1999) reported in his study of 22 forest soils in the Sabie area of the eastern escarpment. The pH in the forest samples is on average 0.28 units lower than the grassland samples. Similarly, Knoepp and Swank (1994) reported a decline in pH of 0.2 units in the topsoil of a southern Appalachian mixed-hardwood stand over a period of twenty years. Morris (1984) also reported a similar decline in topsoil pH of 0.15 units in a *Pinus patula* stand of 27 years in Swaziland.

The impact of afforestation on pH is more pronounced in pH (H_2O) than in pH (KCl), with $p = 0.06$ and $p = 0.28$, respectively. Furthermore, the difference in pH (H_2O) and pH (KCl) (ΔpH) is significantly larger in the grassland samples relative to the forest samples despite a larger CEC_e in the latter. Likewise, Fey *et al.* (1998) noted an afforestation-induced reduction of ΔpH in South African highland soils acidified through land use practices. These data suggest that perhaps a consequent effect of afforestation-induced acidification is an enhancement of positively charged exchange surfaces resulting in the development of anion exchange capacity (AEC). Gubevu (1997), for example, reported an AEC of about 5 $mmol_c kg^{-1}$ for two soils in the eastern escarpment area of South Africa using the compulsive exchange method (Gillman, 1979). Unfortunately, the sorption experiment intended to investigate AEC with respect to NO_3^- in this study was inconclusive; this is discussed further in section 4.2.3.

4.2.2 Organic matter and associated properties

This study, unlike previous studies in the area assessing impacts of afforestation on soil chemical properties (du Toit, 1993; Nowicki, 1997; Sugarman, 1999), included the partially decomposed litter layer (organic O horizon) and subjacent mineral topsoil horizon in the forest sampling. The inclusion of the organic horizon has proved quite informative in the assessment of soil chemical properties in general and particularly in the assessment of soil N form and mobility. Due to the inclusion of the organic horizon in the forest samples there is a marked enhancement in organic C in the forest samples relative to the grassland samples. The enhanced organic matter singularly accounts for the enhanced total N status in the forest samples substantiated by the significant trend between the two variables (organic C and total N) for all samples and by virtue of the dominance of the organic pool as a repository for soil N. The role of organic matter status with respect to N is discussed in more detail in the next section.

Other soil chemical properties affected by the enhanced organic matter status in the forest samples relative to the grassland samples include CEC_e and soil solution EC. Nowicki (1997), for example, reported a median CEC_e of 28.6 $mmol_c kg^{-1}$ for ten forest soils in the eastern escarpment area. The forest samples in the current study, on the other hand, are characterised by a median CEC_e of 44.5 $mmol_c kg^{-1}$, approximately 1.6 times as great as that reported by Nowicki (1997). The significant correlation between CEC_e and organic C content ($r_s = 0.92$, $p < 0.001$) in this study suggests that the enhanced CEC_e in the forest samples results from the enhanced organic matter content. Similarly, Alriksson and Olsson (1995) reported a strong correlation between CEC and C content ($R^2 = 0.98$) for eighteen soils in *Picea abies* (L.) Karst. stands irrespective of soil depth in the A horizon. This relationship has also been substantiated by detailed studies reviewed by Sposito (1989) and McColl and Gressel (1995) which report a positive correlation between cation exchange capacity and soil organic matter content.

Complementing the enhanced CEC_e in the forest relative to the grassland samples, which has been attributed to an enhanced organic matter status, is a depleted EC in the soil solutions of the former. Cations are expected be less mobile in soils with larger CEC as they will be bound to exchange sites and partitioned to the solid phase. Soluble and strongly hydrating cations are strongly held by humus clays and are, therefore, expected to be more concentrated in the

soil solution of soils with lower CEC (McBride, 1994) resulting in an enhancement of EC. Nowicki (1997) reported a median EC of 192 μScm^{-1} for 12 forest soils in the eastern escarpment which is slightly lower than the median EC of forest samples in this study (201 μScm^{-1}), perhaps reflecting seasonal variations. Interestingly, however, Nowicki (1997) reported a significant enhancement in EC in the *forest* soils relative to grassland soils ($p = 0.03$), which is opposite to the trend demonstrated by this study. Comparison of the current study with Nowicki's (1997) lends weight to the interpretation that the depleted EC in the forest samples results, in part, from the enhanced organic matter content.

Organic matter also appears to play a role in buffering Al specifically, and acidity in general in the soils. There is a general positive correlation between organic C content and extractable acidity for all the samples. Furthermore, there appears to be a steeper trend in the relationship between organic C and acidity of the forest samples than in that of the grassland samples, suggesting that for a given amount of organic matter there is expected to be an enhanced acidic character in the forest soils relative to the grassland samples. This observation supports the theory that organic matter plays an important role in Al buffering in acid forest soils (Mulder and Stein, 1994; Ross and Bartlett, 1996).

4.2.3 Nitrogen form and mobility

The forest samples are characterised by significantly higher total N content, soil solution NO_3^- concentration relative to ionic strength and extractable NO_3^- concentration than the grassland samples. As previously discussed, the enhanced total N status of the forest samples is attributed to the inclusion of organic horizons in the sampling which has resulted in an enhanced organic matter status. There is a strong correlation between organic C and total N with $r_s = 0.96$ ($p < 0.001$) and the magnitude of enhancement in the forest samples relative to the grassland samples is virtually the same for both parameters (approximately doubled in each case). Gundersen *et al.* (1998) similarly reported a correlation between the contents of organic matter (kg OM/m^2) and N (g N/m^2) ($r_s = 0.90$ and $p = 0.04$) in the organic layer of five forest stands studied in conjunction with the NITREX project.

Soil solution NO_3^- and NH_4^+ was examined in detail in Chapter 2. Upon first glance there appears to be no difference in NO_3^- concentration between vegetation types. Further inspection, however, reveals an enhanced NO_3^- status relative to ionic strength in the forest samples relative to the grassland samples ($p = 0.06$). This confirms earlier studies in the area

reporting enhanced NO_3^- concentrations in forest soil solutions and streams draining forest catchments compared to those draining grassland catchments (Nowicki, 1997; Fey *et al.*, 1999).

A comparison of soil solution NO_3^- and NH_4^+ concentrations between the current study and Nowicki's (1997) study is presented in Table 4.1.

Table 4.1 A comparison of median soil solution NO_3, NH_4 and NH_4/NO_3 between the current study and Nowicki (1997) where soil solution concentrations are given in mmol l^{-1}.

	Current study			**Nowicki (1997)**		
	Grassland	Forest	p value	Grassland	Forest	p value
NO_3	0.057	0.075	0.57	0.027	0.267	< 0.001
(NO_3/I)[1]	0.021	0.060	0.03	na[2]	na	na
NH_4	0.158	0.093	0.07	0.329	0.302	0.30
NH_4/NO_3	2.8	1.2	0.15	12.0	3.0	< 0.001

[1] NO_3/I is NO_3 per kg of soil normalised with respect to ionic strength
[2] where na is not applicable

In general the trends are the same between the two studies: enhanced NO_3^- concentration in the forest samples (specifically relative to ionic strength), a slight enhancement in NH_4^+ concentration in the grassland samples and a larger NH_4^+/NO_3^- ratio in the grassland samples. The magnitude of the enhancement in the forest samples in this study, however, is not the same as that reported in Nowicki's (1997) study. There are a number of differences between the previous study and the current study that may account for observed differences, including sampling period, sampling strategy and soil texture. Nowicki's (1997) sampling took place in May 1995 and 1996 and, as previously mentioned, the organic horizons were excluded in the forest samples and, furthermore, the soils were in general richer in clay content (median clay content of 25 and 27% for grassland and forest samples, respectively, compared to 2.1 and 4.2% for the current study). The difference in sampling periods could account for seasonal differences specifically and temporal differences in general. Not only is the sampling season (autumn compared to late winter) a consideration, but the antecedent climatic conditions characterising the particular sampling period are also expected to influence soil solution chemistry generally and soil N transformations particularly. The inclusion of organic horizons in this study has been used as explanation of the lower ionic strength (EC) in the

forest samples relative to the grassland samples. The exclusion of organic horizons in the previous study may, in part, account for the different trend in ionic strength, manifested as a higher EC in the forest samples relative to the grassland samples. The increased ionic strength in the forest soil solutions of Nowicki's (1997) study will result in an overstatement of enhancements in ionic concentrations in the forest samples relative to the grassland samples. The enhanced clay content of the soils in the previous study may also contribute to apparent differences in the two studies. A higher clay content implies a larger specific surface area, which might be expected to increase soil solution/solid interactions. The myriad factors influencing soil solution chemistry and soil N transformations attests to the need for further research regarding N form and mobility in forest and grassland soils in the highlands of South Africa before a comprehensive understanding regarding the apparent differences is possible.

Soil solution (per kg of soil) and KCl-extractable mineral N data from this study are compared in Table 4.2.

Table 4.2 Soil solution and 2 M KCl-extractable median NO_3 and NH_4 concentrations for grassland and forest samples for this study where concentrations are given in mmol kg^{-1}. ΔNO_3 and ΔNH_4 are the difference in extractable and soil solution NO_3 and NH_4, respectively.

	Grassland	Forest	p value
NO_3	0.019	0.053	0.12
NH_4	0.046	0.063	0.89
Extractable-NO_3	0.149	0.268	0.06
Extractable-NH_4	0.261	0.447	0.15
ΔNO_3	0.13	0.21	0.12
ΔNH_4	0.21	0.38	0.25

There is significantly more extractable NO_3^- in the forest samples compared to the grassland samples ($p = 0.06$). Similarly, there is an apparent enhancement in extractable NH_4^+ concentration in the forest samples relative to the grassland samples, although not significant ($p = 0.15$). In general, there is an enhancement of mineral N in the KCl extracts compared to the soil solution extracts. For NH_4^+, this is primarily attributed to exchangeable NH_4^+ associated with the solid phase of the soil. The larger ΔNH_4^+ (difference in extractable and soil solution NH_4^+) in the forest samples relative to the grassland samples ($p = 0.25$) reflects

the enhanced CEC_e in the former relative to the latter. Matschonat and Matzner (1995) similarly reported that the NH_4^+ storage capacity of two haplic podzols from the German Fichtelgebirge is mainly a function of CEC.

Table 4.2 also indicates that, similarly to ΔNH_4^+, there is a larger ΔNO_3^- in the forest samples relative to the grassland samples ($p = 0.12$). The enhanced NO_3^- concentrations in the KCl extracts relative to the saturated paste extracts coupled with ΔpH data (section 4.2.1) motivated a NO_3^- sorption experiment intended at assessing the potential of NO_3^- storage on anion exchange sites in the soil. The experiment yielded evidence of NO_3^- sorption at low treatments, but was inconclusive at higher treatments. There is evidence of AEC in acidic soils (both under grassland and forest) in the eastern escarpment area of South Africa. Gubevu (1997), as mentioned earlier, determined an AEC of approximately 5 $mmol_c kg^{-1}$ for two soils in the area, which is comparable in magnitude to values obtained for similar soils determined by the compulsive exchange method (Gillman, 1979; Parfitt, 1980). Gillman (1979), for example, reported AEC values between 0.1 and 0.2 $cmol_c kg^{-1}$ for topsoils and between 0.2 and 3.7 $cmol_c kg^{-1}$ for subsoils. The study conducted by Gubevu (1997), however, excluded the partially decomposed litter layer and subjacent organic layers in the forest samples and the AEC that was determined was attributed primarily to the presence of sesquioxides. The contribution of organic matter, particularly associated with pine litter, to AEC has not been examined in the area. The possibility of protonated and/or metal complexed humic substances producing positive surface charge needs further consideration.

In order to assess and examine major processes associated with soil N form and mobility and differences between the two vegetation types, two N mineralisation experiments were conducted. An aerobic N mineralisation incubation was conducted with and without added $(NH_4)_2SO_4$ to assess net rates of nitrification with and without unlimited nitrifiable substrate. Calculating net rates of nitrification (mmol NO_3^- kg $soil^{-1} day^{-1}$) revealed a significant enhancement in the forest samples relative to the grassland samples ($p = 0.05$) when not treated with $(NH_4)_2SO_4$. The enhanced rate in the forest soils is not related to total N content nor to organic matter content. There is a slight negative correlation between rate of net nitrification and C/N for all samples and a stronger correlation for the forest samples alone, suggesting that C/N may be related to rate of nitrification in the forest samples. In general, there is a positive correlation between net rate of nitrification in the untreated samples and soil solution and KCl-extractable NO_3^- (Chapter 3), suggesting that the enhanced NO_3^- concentrations in forest samples results from an enhanced net rate of nitrification.

The addition of $(NH_4)_2SO_4$ resulted in no significant difference in rate of nitrification between vegetation types. The difference in net rate of nitrification between treated and untreated samples (Δ nitrification) revealed a generally negligible or even slightly inhibiting effect of $(NH_4)_2SO_4$ amendment in the forest samples and a slight enhancing effect in the grassland samples. The inhibiting effect of $(NH_4)_2SO_4$ on the forest net nitrification rates is attributed to the acidifying effect of the amendment (Tisdale *et al.*, 1985) coupled with the overall higher acidity status in the forest samples relative to the grassland samples. This trend is substantiated by the positive correlation between Δ nitrification and pH ($r_s = 0.77$, $p = 0.002$).

The second N mineralisation experiment consisted of water-logging samples and incubating at elevated temperatures (40°C) for seven days and measuring the NH_4^+ produced relative to control samples that were frozen for the same time period. The rate of mineralisation (mmol N kg^{-1} day^{-1}) is significantly enhanced in the forest samples relative to the grassland samples ($p = 0.04$). This enhancement in the forest samples is singularly attributed to the enhanced total N content in the forest samples relative to the grassland samples; the correlation between rate of mineralisation (per kg of soil) and total N is characterised by $r_s = 0.94$ ($p < 0.001$). Expressing the rate of mineralisation relative to the organic carbon content (mmol N $kgOC^{-1}$ day^{-1}) eliminates the difference in rates between the vegetation types ($p = 0.57$), suggesting that although the total amount of N mineralised per kg of soil is considerably larger in the forest samples, the fraction of total N that is mineralisable is virtually the same between vegetation types.

As stated earlier, there is a weak relationship between net rate of nitrification (untreated) and C/N ratio (particularly for the forest samples). Similarly, there is a weak negative correlation between net rate of mineralisation (anaerobic incubation mmol N $kgOC^{-1}$ day^{-1}) and C/N ratio ($r_s = -0.52$, $p = 0.07$) for all samples and a more significant trend for the forest samples alone ($n = 7$, $r_s = -0.75$, $p = 0.05$). The relationship between C/N ratio and N mineralisation in general and nitrification in particular is commonly quoted for forest ecosystems (e.g. Van Miegroet *et al.*, 1989; Emmett *et al.*, 1998; Gundersen *et al.*, 1998). The substantiated link between mineralisation and nitrification rates and C/N is, however, only consistent for C/N in the forest floor and not in the mineral soil. Gundersen *et al.* (1998) reported, in a synthesis of the NITREX project, that mineral soil C/N is not correlated to mineralisation and the mineral pool might not need to change for N saturation to occur. Since the forest sampling in this study consisted of a combination of the organic forest floor soil horizon and the topsoil

mineral horizon, differences in mineralisation and N status might consequently be obscured that may otherwise have been apparent had the horizons been sampled separately.

4.2.4 Soil properties that discriminate according to vegetation

Table 4.3 lists the soil properties for which there is a significant difference between vegetation types ($p < 0.10$), including p values assessing the significance of the difference in each case, as well as the median values and ranges for the forest and grassland samples. The soil properties that discriminate between vegetation types can be broadly grouped into four general categories: **acidity status** including soil pH, extractable acidity, acid saturation and ΔpH; **organic matter status** including organic carbon, CEC_e, extractable cations, EC and soil solution ion concentrations; **nitrogen status** including total N, NO_3^-/I, extractable NO_3^-, anaerobic mineralisation rate and net nitrification rate (untreated); and **soil texture** including clay and sand content. These four categories are not, however, mutually exclusive. There is, in fact, some overlap between them, for example, the relationship between organic matter and total N status has been discussed at length, as has the relationship between acidity and organic matter. The soil properties listed in Table 4.3 were used in a forward step-wise discriminant analysis in order to determine which variables discriminate between the vegetation types most effectively. The discriminant analysis identified the following soil properties in order of efficiency: acidity, soil solution Ca^{2+}, ΔpH, extractable NO_3^-, soil solution NO_3^-/I, extractable Na^+ and rate of net nitrification as calculated from the aerobic (control) incubation.

The top three variables identified by the discriminant analysis (acidity, soil solution Ca^{2+} and ΔpH) provide means with which to illustrate the differences in vegetation type. Figure 4.1 shows a scatterplot of the top three discriminating variables and effectively illustrates the differences between the forest and grassland soils in three-dimensional space.

Table 4.3 Mann Whitney U test results for variables for which there is a significant difference ($p < 0.10$) between grassland and forest soil samples. Numbers in parentheses following the variables indicate order in which entered into forward step-wise discriminant analysis.

Variable	p value	Range (f)[1]	Median (f)	Range (g)[2]	Median (g)
pH (H_2O)	0.063	4.28 - 4.89	4.68	4.55 - 5.28	4.96
pH (SPE)	0.086	4.83 - 6.23	6.01	6.02 - 6.72	6.16
ΔpH[3] (3)	0.016	0.6 - 0.8	0.7	0.7 - 1.0	0.9
Extractable acidity ($mmol_c kg^{-1}$) (1)	0.003	20.9 - 52.8	41.9	5.2 - 17.8	8.6
Acid saturation (%)	0.032	77 - 94	86	51 - 84	80
Organic C (%)	0.004	3.4 - 9.8	6.3	1.0 - 3.5	2.8
CEC_e ($mmol_c kg^{-1}$)	0.003	26.9 - 60.9	44.5	6.5 - 22.2	13.4
Extractable Mg ($mmol_c kg^{-1}$)	0.086	0.1 - 7.3	1.1	0.0 - 1.0	0.3
Extractable Na ($mmol_c kg^{-1}$) (6)	0.010	0.5 - 1.2	0.7	0.2 - 0.6	0.3
EC (μScm^{-1})	0.007	184 - 243	201	220 - 344	278
NH_4 ($mmol_c l^{-1}$)	0.074	0.05 - 0.20	0.09	0.10 - 0.34	0.16
K ($mmol_c l^{-1}$)	0.007	0.30 - 0.63	0.42	0.48 - 1.38	0.77
Ca ($mmol_c l^{-1}$) (2)	0.032	0.00 - 0.34	0.33	0.28 - 0.68	0.36
F ($mmol_c l^{-1}$)	0.063	0.02 - 0.06	0.03	0.03 - 0.06	0.04
SO_4 ($mmol_c l^{-1}$)	0.022	0.14 - 0.46	0.17	0.24 - 0.59	0.39
Total N (%)	0.010	0.14 - 0.48	0.33	0.05 - 0.17	0.14
NO_3/I[4] (5)	0.032	0.02 - 0.18	0.060	0.01 - 0.09	0.021
Extractable NO_3 ($mmol\,kg^{-1}$) (4)	0.063	0.14 - 0.53	0.27	0.08 - 0.41	0.15
Aerobic NO_3 ($mmol\,kg^{-1}day^{-1}$) (7)	0.046	0.02 - 0.10	0.036	0.00 - 0.10	0.006
Anaerobic NH_4 ($mmol\,kg^{-1}day^{-1}$)	0.004	0.22 - 0.60	0.34	0.04 - 0.23	0.16
Clay content (%)	0.018	2.0 - 8.2	4.2	1.1 - 3.8	2.1
Sand content (%)	0.015	77.2 - 91.0	83.4	85.2 - 95.9	92.0

[1] where *f* denotes forest
[2] where *g* denotes grassland
[3] $\Delta pH = pH\ (H_2O) - pH\ (KCl)$
[4] where NO_3/I is soil solution NO_3 ($mmol\ kg^{-1}$)/ ionic strength ($mmol\ kg^{-1}$)

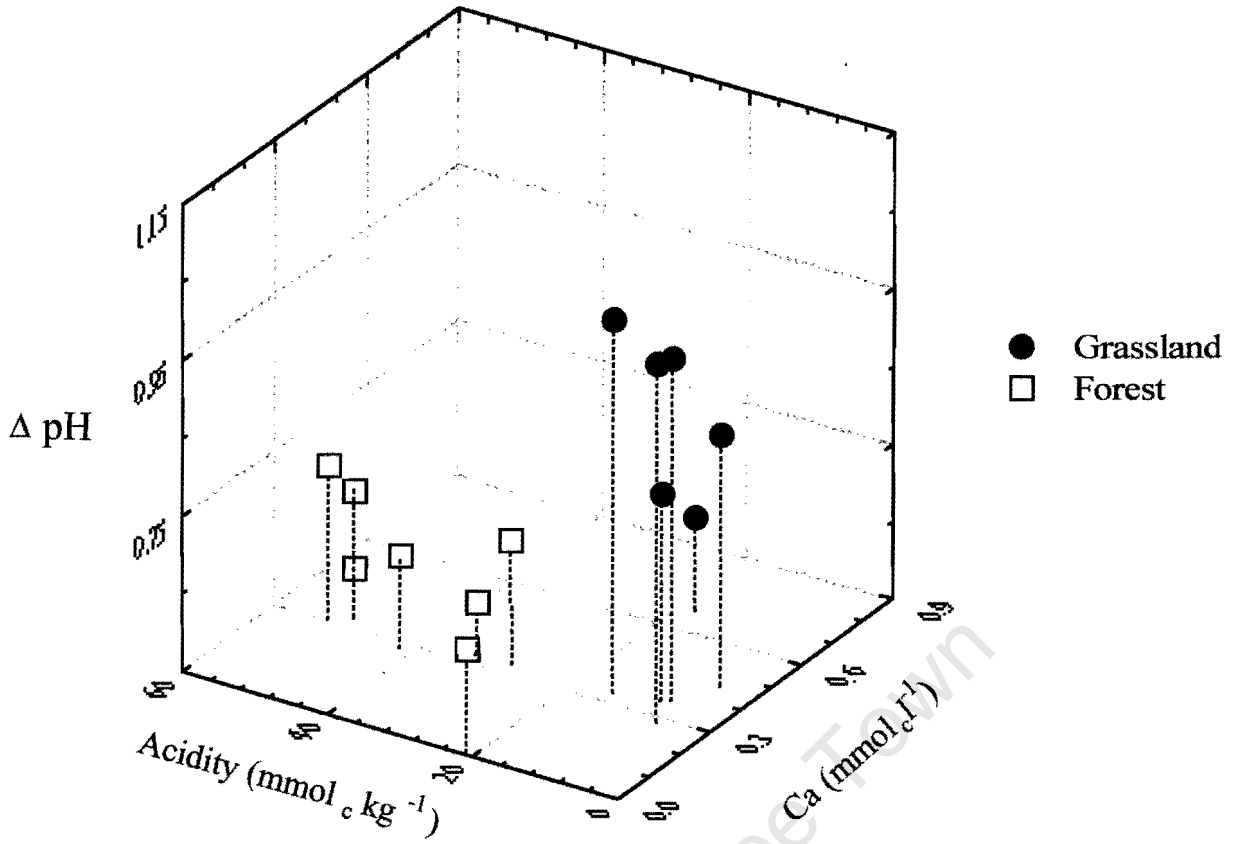


Figure 4.1 Relationship between the top three discriminating variables [acidity vs. Ca vs. ΔpH (defined as pH(H_2O) – pH (KCl))] which provide a soil chemical differentiation of forest and grassland soils.

4.3 Practical implications

One of the most serious consequences of excess mineral N in soils is N leaching and contamination of nearby surface and groundwater. Nitrate leaching is of particular concern compared to NH_4^+, since NO_3^- is a strong acid anion and is, therefore highly mobile. Nitrate contamination of drinking water is a serious concern as it can be reduced to NO_2^- in the gastrointestinal tract (DWAF, 1993). Excess NO_3^- leaching from soils into streams might also cause eutrophication (Emmett *et al.*, 1993). Current research (Fey *et al.*, 1999) into stream water chemical composition has revealed an average four-fold enhancement of NO_3^- concentration in streams draining forest catchments compared to those draining comparable grassland catchments (0.010 compared to 0.043 mmol l^{-1}) in the eastern escarpment area of South Africa. These values of NO_3^- are of little environmental consequence, however, since the DWAF guideline for NO_3^- in drinking water is an order of magnitude higher (0.4 mmol l^{-1}) than the average concentration for stream waters draining forest catchments.

Although environmentally benign, the enhanced NO_3^- concentrations in the forest soils and corresponding stream waters seems to serve a potentially useful function in flagging the impact of afforestation, particularly the loss of base cations and accompanying soil and water acidification. The removal of base cations and the subsequent leaching of H^+ and Al^{3+} associated with NO_3^- leaching may pose a serious threat of long-term acidification of surface waters in poorly buffered catchments and may also result in nutrient imbalances in the forest ecosystem.

4.4 Recommendations for future work

The results of this study emphasise the importance of organic matter accumulation in the forest floor coupled with C/N ratio in influencing N mineralisation and resulting in an enhanced NO_3^- status in forest soils. Future studies are necessary to confirm these findings and to gain a better understanding of the relative contributions of enhanced interception of the forest canopy of atmospheric N and of microbiological activity in influencing overall N status. In order to obtain reproducible and accurate rates of mineralisation and an indication of spatial and temporal variability, *in situ* mineralisation experiments could be conducted. These experiments, coupled with detailed throughfall data, would make ecosystem mass balance calculations possible and help elucidate changes in N status resulting from afforestation of natural grasslands in the eastern escarpment area of South Africa. Whole regolith studies would also be valuable, preferably coupled with accurate hydrological data including relative magnitude of surface runoff and throughflow of waters.

Other interesting aspects of this study that warrant further study include the possibility of AEC and NO_3^- sorption capacity and the role of organic matter in acidity and Al buffering. It would be useful to determine the AEC directly for the soil profile and concurrently measure extractable NO_3^- concentrations in order to obtain an indication of the NO_3^- retention capacity of the soils. Further characterisation of organic matter, particularly associated with the forest floor accumulation of biomass, is needed to assess the role of humic substances in buffering soils and in controlling Al solubility.

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Appendix A. Graskop and Kaapsehoop sampling site details

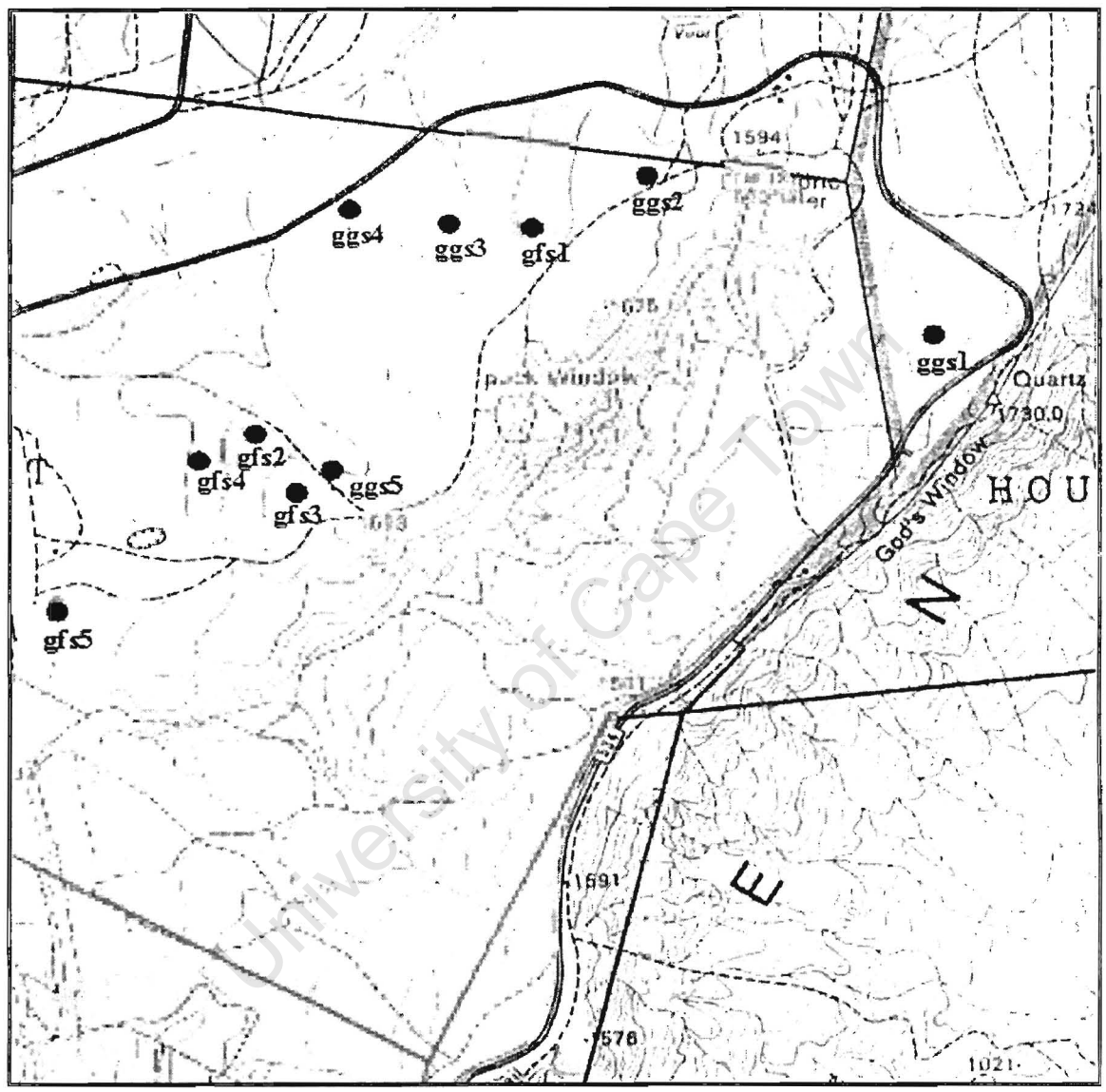

Figure A.1 Graskop sampling map modified after 1:50 000 topographic map 2430DD Graskop (South African Surveys and Land Information, 1986). Map area extends from 24°51' to 24°54' S latitude and from 30°51' to 30°54' E longitude and shaded areas ▒ denote selected forested areas.

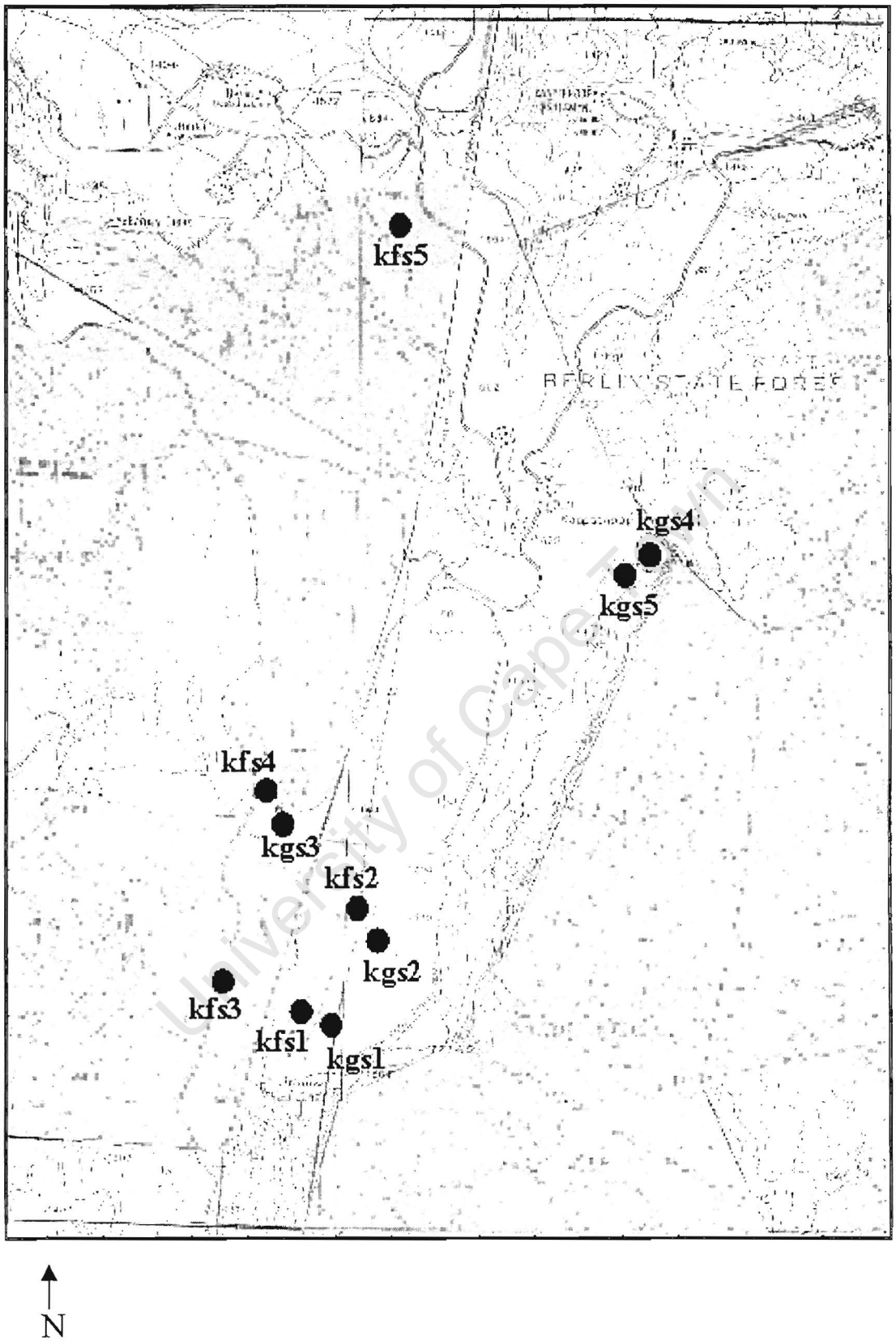

Figure A.2 Kaapsehoop sampling map modified after 1:50 000 topographic maps 2530DA Ngodwana (1988) and 2530DB Kaapsehoop (1984) (South African Surveys and Land Information, 1986). Map area extends from 25°33' to 25°39' S latitude and from 30°43' to 30°48' E longitude and shaded areas denote selected forested areas.

Appendix B. Analytical methods and appraisal

This Appendix contains a description of analytical methods used during this study and presents a critical appraisal of the precision of the methods and the quality of the data. Replicate analyses were made and evaluated in terms of mean, standard deviation (SD) and relative standard deviation (RSD), serving as an index of data quality.

B.1 Sample preparation

Samples were shipped from the study area via courier to the University of Cape Town within 3 days of sampling. Approximately three-quarters of each sample was air-dried, crushed to pass a 2-mm mesh and stored in 1-litre wide mouthed plastic bottles. The remaining approximate one-quarter of each sample was passed through a 2-mm sieve and refrigerated in order to maintain field-moist conditions and to inhibit microbial transformations. Refrigerated samples were used for 2 M KCl-extractable NO_3^- and NH_4^+ analyses and N mineralisation experiments. The moisture content of the air-dried and field-moist samples was determined gravimetrically by heating at 110°C for at least 12 hour. The moisture content was subsequently used as a correction factor for calculating dry mass.

B.2 pH measurement in deionized water and 1 M KCl

Theory

The intensity of acidic or basic character of a solution is governed by pH, which is a measure of the H^+ activity in solution. Pure water ionises slightly to form H^+ and OH^- ions and is governed by the following equilibrium equation:

$$\alpha_{H^+}\alpha_{OH^-} = K_w = 1.01 \times 10^{-14} \text{ at } 25°C \qquad (1)$$

where α_{H^+} = activity of H^+ ($mmol_c l^{-1}$)
α_{OH^-} = activity of OH^- ($mmol_c l^{-1}$)
K_w = equilibrium dissociation constant of water.

Transforming the above equation (1) logarithmically results in the following pH relationship:

$$pH + pOH = pK_w = 14 \text{ at } 25°C \qquad (2)$$

where $pH = -\log_{10} \alpha_{H^+}$
$pOH = -\log_{10} \alpha_{OH^-}$
$pK_w = -\log_{10} K_w$.

pH was measured potentiometrically with a standard glass electrode and reference electrode. An electromotive force is produced in the glass electrode in response to the activity of H^+ ions in solution. The reference electrode provides a constant potential by way of a salt bridge that produces a liquid junction potential (Standard Methods, 1995).

Method

The pH of the samples was measured using three different techniques. Firstly, a mixture of 10g of sample and 25ml of deionized water was allowed to equilibrate for thirty minutes. The pH of the supernatant was measured using a Metrohm 691 pH meter. The procedure was repeated using 1 M KCl solution instead of deionized water as the second technique. The pH of the saturated paste extract (SPE) was measured as the third technique (section B.3). The pH meter was standardised with buffers of known pH (pH 4.02 and 7.00, respectively) prior to each set of pH measurements.

One M KCl is employed because the large concentration of a neutral salt eliminates a suspension effect upon the liquid junction potential. A modification in K^+ and Cl^- mobility across the salt bridge of the reference electrode due to interaction with the electrical double layer of charged clay mineral surfaces occurs if a concentrated salt is not used, this is termed suspension effect (McBride, 1994).

Analysis and data appraisal

The precision of the data produced by pH measurements was evaluated according to the reproducibility of the results. Tables B.1, B.2 and B.3 shows the repeats of the measurements made, the arithmetic mean, standard deviation (SD) and relative standard deviation (RSD). The RSD of pH (KCl) measurements were all well below 2% indicating good precision; for pH (H_2O) the RSD of all samples were within 4%, which is also satisfactory; for pH (SPE) the RSD is less precise than the other two methods with RSD values within 5%. Overall the precision of the data is acceptable. The pH (KCl) results are the most precise perhaps due to the consistent ionic strength produced using a concentrated neutral salt that prevents a dilution effect (Alloway, 1995). In general, the pH (SPE) results are less consistent on account of uncertainties associated with possible CO_2 degassing resulting from the extraction procedure and an equilibrium delay following the separation of solution and solid. It may have been more instructive to measure the pH in the saturated paste itself, rather than the extract (Rowell, 1994).

Table B.1 Precision of pH (KCl) data.

Sample	pH (KCl)	pH (KCl)	mean	SD	RSD(%)
ggs1	4.22	4.15	4.19	0.05	1.18
ggs2	3.88	3.82	3.85	0.04	1.10
ggs3	4.11	4.06	4.09	0.04	0.87
ggs4	4.20	4.15	4.18	0.04	0.85
ggs5	4.22	4.18	4.20	0.03	0.67
gfs1	3.98	3.95	3.97	0.02	0.54
gfs2	3.95	3.92	3.94	0.02	0.54
gfs3	3.63	3.58	3.61	0.04	0.98
gfs4	4.07	4.02	4.05	0.04	0.87
gfs5	3.77	3.74	3.76	0.02	0.56
kgs1	4.30	4.27	4.29	0.02	0.50
kgs2	4.28	4.27	4.28	0.01	0.17
kgs3	4.31	4.30	4.31	0.01	0.16
kgs4	3.72	3.74	3.73	0.01	0.38
kgs5	4.14	4.13	4.14	0.01	0.17
kfs1	4.20	4.20	4.20	0.00	0.00

Table B.1 Precision of pH (KCl) data (continued).

Sample	pH (KCl)	pH (KCl)	mean	SD	RSD(%)
kfs2	4.07	4.06	4.07	0.01	0.17
kfs3	2.72	2.72	2.72	0.00	0.00
kfs4	3.83	3.82	3.83	0.01	0.18
kfs5	4.00	4.01	4.01	0.01	0.18

Table B.2 Precision of pH (H_2O) data.

	pH (H_2O)	pH (H_2O)	pH (H_2O)	mean	SD	RSD(%)
ggs1	4.72	4.72		4.72	0.00	0.00
ggs2	4.78	4.97	4.83	4.86	0.10	2.03
ggs3	5.12	4.77	4.74	4.76	0.21	4.44
ggs4	5.05	5.05		5.05	0.00	0.00
ggs5	5.25	5.21		5.23	0.03	0.54
gfs1	4.59	4.57		4.58	0.01	0.31
gfs2	4.70	4.67		4.69	0.02	0.45
gfs3	4.33	4.22		4.28	0.08	1.82
gfs4	4.78	4.74		4.76	0.03	0.59
gfs5	4.45	4.48		4.47	0.02	0.48
kgs1	5.26	5.29		5.28	0.02	0.40
kgs2	5.23	5.24		5.24	0.01	0.14
kgs3	5.03	5.00		5.02	0.02	0.42
kgs4	4.65	4.45	4.54	4.55	0.10	2.20
kgs5	4.70	4.70		4.70	0.00	0.00
kfs1	4.85	4.92		4.89	0.05	1.01
kfs2	4.75	4.64	4.66	4.68	0.06	1.25
kfs3	3.27	3.25		3.26	0.01	0.43
kfs4	4.49	4.49		4.49	0.00	0.00
kfs5	4.92	4.93		4.93	0.01	0.14

Table B.3 Precision of pH (SPE) data.

Sample	pH(SPE)	pH(SPE)	mean	SD	RSD(%)
ggs1	7.12	6.76	6.94	0.25	3.67
ggs2	5.98	6.12	6.05	0.10	1.64
ggs3	6.40	6.08	6.24	0.23	3.63
ggs4	6.96	6.47	6.72	0.35	5.16
ggs5	6.84	6.45	6.65	0.28	4.15
gfs1	5.21	5.23	5.22	0.01	0.27
gfs2	6.23	6.22	6.23	0.01	0.11
gfs3	4.90	4.76	4.83	0.10	2.05
gfs4	6.06	6.12	6.09	0.04	0.70
gfs5	5.68	5.76	5.72	0.06	0.99
kgs1	6.11	6.03	6.07	0.06	0.93
kgs2	6.03	5.92	5.98	0.08	1.30
kgs3	6.23	6.18	6.21	0.04	0.57
kgs4	6.09	5.95	6.02	0.10	1.64

Table B.3 Precision of pH (SPE) (continued).

Sample	pH(SPE)	pH(SPE)	mean pH	SD	RSD(%)
kgs5	6.22	6.10	6.16	0.08	1.38
kfs1	6.04	5.98	6.01	0.04	0.71
kfs2	6.12	6.06	6.09	0.04	0.70
kfs3	3.54	3.49	3.52	0.04	1.01
kfs4	5.22	5.09	5.16	0.09	1.78
kfs5	5.46	5.33	5.40	0.09	1.70

B.3 Saturated paste extracts

Saturated paste extracts were used to approximate the soil solution according to (Rhoades, 1982). Approximately 250-350 g of sample and sufficient distilled water to reach saturation were made. The pastes were allowed to equilibrate for at least 12 hours and kept covered to prevent evaporation. The paste extract was then obtained by suction through a Whatman # 50 filter paper on a Buchner funnel. The extracts were used for the following analyses.

B.3.1. Electrical conductivity (EC)

Theory

Conductivity of an aqueous solution is the reciprocal of resistance, and hence, a measure of the solution's capacity to carry an electric current. The tendency to conduct an electric current is correlated to the presence of ions in solution and, therefore, is often used as an indication of salinity or total dissolved solids.

Method

Electrical conductivity was measured with a CRISON micro CM 2201 that consists of a conductivity cell and a temperature probe. EC was reported in μScm^{-1}. A calibration was done prior to every set of measurements with a standard 0.01 M KCl solution.

Analysis and data appraisal

The reproducibility of the EC measurements is presented in Table B.4. In general, there is good reproducibility of data with the majority of RSD within 2%. There are, however, some sub-optimal duplicates namely, ggs1 (8.4%), ggs3 (10.6%), ggs4 (5.8%) and ggs5 (8.2%). Standard Methods (1995) states that most problems in acquiring good data are related to electrode fouling. It is possible for salts to precipitate on the electrode and cause interference. Despite the spurious results for the above samples, the results were considered satisfactory for the purposes of this study.

Table B.4 Precision of EC data.

Sample	EC (μScm^{-1})	EC (μScm^{-1})	Mean EC	SD	RSD(%)
ggs1	240	213	227	19.1	**8.4**
ggs2	288	288	288	0.0	0.0
ggs3	289	336	313	33.2	**10.6**
ggs4	256	278	267	15.6	**5.8**
ggs5	324	364	344	28.3	**8.2**

Table B.4 Precision of EC data (continued)

Sample	EC (μScm^{-1})	EC (μScm^{-1})	Mean EC	SD	RSD(%)
gfs1	204	206	205	1.4	0.7
gfs2	185	184	184	0.7	0.4
gfs3	188	186	187	2.0	1.1
gfs4	225	224	225	0.7	0.3
gfs5	194	197	196	2.1	1.1
kgs1	246	243	245	2.1	0.9
kgs2	189	189	189	0.4	0.2
kgs3	256	256	256	0.0	0.0
kgs4	220	220	220	0.0	0.0
kgs5	146	145	145	0.8	0.5
kfs1	204	198	201	4.5	2.3
kfs2	243	243	243	0.0	0.0
kfs3	303	293	298	7.1	2.4
kfs4	234	228	231	4.2	1.8
kfs5	283	279	281	2.8	1.0

B.3.2. Ion chromatography (IC)

Theory

Ion chromatography is an analytical technique for the separation and determination of ions in solution. An eluent is passed through a solid stationary phase consisting of ion exchange resin beads packed into a cylindrical column. A high-pressure pump is required to force the eluent through the column. In-line detection of the separated ions of interest is achieved by monitoring conductivity, with chemical suppression of the eluent.

Method

Analysis of anions and cations in the saturated paste extracts were carried out with a Dionex DX300 series suppressed IC system coupled to an AI-450 chromatography software package. IC was used to determine the major cation (Li^+, Na^+, K^+, Ca^{2+}, Mg^{2+} and NH_4^+) and anion (F^-, Cl^-, NO_2^-, Br^-, NO_3^-, PO_4^{3-} and SO_4^{2-}) concentrations in the saturated paste extracts. The extracts were passed through 0.45μm Millipore filter membranes. Samples were diluted such that their electrical conductivities were below $100\mu Scm^{-1}$.

Analysis and data appraisal

The precision of the data is presented in Table B.5. Generally, the data is reproducible with the RSD < 10%. There are a few exceptions to this, but they occur when ion concentration is low and approaching detection limits.

Table B.5 Precision of IC data (not corrected for dilution).

Sample		Na^+	NH_4^+	K^+	Mg^{2+}	Ca^{2+}	F^-	Cl^-	NO_3^-	SO_4^{2-}
	mg/l	7.0	1.2	11.1	1.2	4.5	0.2	17.0	1.1	6.1
	mg/l	5.8	0.8	10.5	1.3	4.5	0.3	17.5	1.2	6.4
ggs3	mean	6.4	1.0	10.8	1.3	4.5	0.3	17.2	1.1	6.2
	SD	0.9	0.2	0.5	0.1	0.0	0.0	0.4	0.0	0.2
	RSD(%)	13.4	22.6	4.3	6.8	0.2	7.3	2.1	2.2	3.4
	mg/l	5.3	1.3	6.5	0.9	2.3	0.2	12.0	11.0	2.2
	mg/l	5.8	1.6	6.5	0.7	1.8	0.3	12.8	12.2	2.1
kgs3	mean	5.6	1.5	6.5	0.8	2.0	0.3	12.4	11.6	2.2
	SD	0.4	0.2	0.0	0.1	0.3	0.0	0.5	0.8	0.1
	RSD(%)	6.5	13.8	0.0	14.3	15.7	13.0	4.2	7.2	3.1
	mg/l	5.3	0.7	2.7	1.7	2.3	0.3	8.7	0.0	9.8
	mg/l	5.4	0.8	2.8	1.6	2.2	0.4	9.2	0.0	10.4
kfs3	mean	5.4	0.7	2.7	1.7	2.2	0.4	8.9	0.0	10.1
	SD	0.1	0.0	0.0	0.1	0.1	0.0	0.4	0.0	0.4
	RSD(%)	1.4	0.9	1.6	4.7	4.7	6.4	4.3	na	3.8

Solutions are electrically neutral - the sum of the charge associated with the anions is equivalent to that associated with the cations. If the charges do not balance, there is an indication that one or more of the analyses is fraught with error. Charge balances were calculated and are presented in Table B.6. Although some of the charge balances are out by more than 20%, it was attributed to low ionic strength solutions (EC data is presented in Table B.4), which results in slight differences in ion concentrations producing exaggerated differences when expressed as a percentage. For all but one sample there is an excess of cations relative to anions. This was interpreted as a general deficiency in anions, particularly dissolved organic matter, which is expected to contribute to the soil solution anion suite in view of large organic carbon content in the solid phase. Specifically samples ggs1 and kfs3, which have an organic carbon content of 10.6 and 8.2%, respectively. The contribution of alkalinity (HCO_3, CO_3) was disregarded based on the acidity and pH of the soil solution. In view of the low ionic strength of the solutions and the absence of DOM data, the percentage difference in charge balance was deemed acceptable for this study.

Table B.6 Charge balance for IC data.

Sample	ggs1	ggs2	ggs3	ggs4	ggs5	gfs1	gfs2	gfs3	gfs4	gfs5
Cations	3.7	2.0	3.1	2.5	2.7	1.8	1.4	1.7	1.7	1.9
Anions	1.4	2.0	1.9	1.7	2.5	1.5	1.2	1.2	1.6	1.3
Cations – Anions	2.2	0.0	1.1	0.7	0.2	0.3	0.2	0.5	0.1	0.6
Ions	5.1	4.0	5.0	4.2	5.3	3.3	2.7	2.8	3.2	3.2
Cation excess (%)	**44.3**	0.8	**22.6**	17.8	3.6	10.1	6.8	18.5	4.0	17.4

Table B.6 Charge balance of IC data (continued)

Sample	kgs1	kgs2	kgs3	kgs4	kgs5	kfs1	kfs2	kfs3	kfs4	kfs5
Cations	2.6	1.6	2.0	1.7	1.1	1.1	2.0	2.8	1.9	2.6
Anions	1.7	1.1	1.8	1.5	0.9	1.2	1.8	1.4	1.7	2.0
Cations – Anions	0.9	0.5	0.2	0.2	0.2	-0.1	0.2	1.3	0.2	0.6
Ions	4.4	2.7	3.8	3.2	2.1	2.4	3.9	4.2	3.6	4.6
Cation excess (%)	20.7	17.1	5.5	7.0	9.5	-4.8	5.6	**32.0**	5.8	13.0

B.3.3. Inductively coupled plasma mass spectroscopy (ICP-MS)

Theory

Inductively coupled mass spectrometry (ICP-MS) is an analytical technique that determines the concentration of trace elements in a sample. Samples can be in liquid form and solids can be nebulised. Analyses can be semi-quantitative or quantitative. A sample is introduced into a stream of Ar plasma, which induces the subsequent vaporisation and ionisation. The ions are then passed through a mass spectrometer where they are separated according to mass/charge ratio. A detector measures the analyte ions as they pass through the mass spectrometer.

Method

Quantitative analyses of the soil solutions (saturated paste extracts) were determined using an Elan 6000 ICP-MS. The extracts were diluted such that their electrical conductivities were below 100 µS/cm. The soil extracts were diluted further with 2% HNO_3 to a 1:1 ratio. A total of 15 ml diluted sample was analysed for Li, Al, Cr, Mn, Fe, Ni, Cu, Zn, As, Se, Rb, Sr, Cd, Cs, Ba, Pb and U.

Analysis and data appraisal

The precision of the data for selected elements is presented in Table B.7. Generally, the data is reproducible with the RSD < 20%. There are a few exceptions to this; most notably Cu in sample gfs3 is characterised by a RSD of 80% strongly suggesting contamination.

Table B.7 Precision of ICP-MS data for selected elements.

Sample		Al	Mn	Ni	Cu	Zn
	µg/l	153.86	242.91	2.58	9.67	17.13
	µg/l	150.23	246.18	2.87	9.71	17.80
	µg/l	184.67	248.28	2.82	11.29	23.96
ggs3	µg/l	183.41	246.14	2.73	10.70	23.25
	mean	168.04	245.88	2.75	10.34	20.54
	SD	18.54	2.22	0.13	0.79	3.57
	RSD (%)	11.03	0.90	4.60	7.66	17.37

Table B.7 Precision of ICP-MS data for selected elements (continued).

Sample		Al	Mn	Ni	Cu	Zn
	µg/l	586.09	123.66	3.21	92.61	31.61
	µg/l	562.69	120.98	3.37	90.48	31.32
	µg/l	855.13	136.88	3.46	16.47	50.79
gfs3	µg/l	842.42	136.44	3.63	16.24	50.74
	mean	711.58	129.49	3.42	53.95	41.12
	SD	158.79	8.35	0.18	43.42	11.14
	RSD (%)	22.31	6.45	5.22	**80.48**	27.10
	µg/l	2408.62	258.01	7.30	23.40	52.02
	µg/l	2504.58	256.81	6.91	23.32	52.01
	µg/l	2413.39	253.37	7.55	15.96	57.29
kfs3	µg/l	2472.23	256.81	7.53	15.63	56.83
	mean	2449.70	256.25	7.32	19.58	54.54
	SD	46.64	2.00	0.30	4.37	2.92
	RSD (%)	1.90	0.78	4.03	22.32	5.35

B.4 Extractable cations

B.4.1. 1 M KCl-extractable acidity (Al)

Theory

Extractable acidity is a quantitative measurement of acidic cations associated with cation exchange sites. In general, it is an aggregate property quantifying an extract's capacity to neutralise a strong base to a designated pH end-point. It is the H^+ ions present in the extract resulting from the hydrolysis of acidic species. The majority of acidic cations associated with exchange surfaces in soils are various hydrolysed forms of Al (e.g. Al^{3+}, $Al(OH)^{2+}$, $Al(OH)_2^+$) and to a lesser extent H^+. Except in very acid soils, exchangeable H^+ is found only in small amounts because clay minerals react with exchangeable H^+ to produce exchangeable Al (Rowell, 1994). The hydrolysis of Al produces acidity according to the following equations:

$$Al^{3+} + H_2O \rightarrow Al(OH)^{2+} + H^+$$
$$Al(OH)^{2+} + H_2O \rightarrow Al(OH)_2^+ + H^+$$
$$Al(OH)_2^+ + H_2O \rightarrow Al(OH)_3 + H^+$$

The complete hydrolysis of 1 mole of Al^{3+} results in the production of 3 moles of H^+.

Method

Extractable acidity was determined according to Thomas (1982). A mixture of 2.5 g of sample and 25 ml 1 M KCl was shaken for 24 hours. The mixture was centrifuged for 5 minutes and filtered through a Whatman #1 qualitative filter. A 10 ml aliquot was titrated potentiometrically with standard 0.01 M NaOH solution to an end-point of pH 8.3 (phenolphthalein end-point). A blank of 25 ml 1 M KCl solution was titrated with the standard 0.01 M NaOH solution and used as a correction factor. The volume of NaOH needed

to neutralise the acidity (corrected for by the result obtained using the blank) was recorded and the number of moles of NaOH was calculated. The following calculation was used to calculate acidity in $mmol_c\,kg^{-1}$ of soil:

$concentration_{NaOH}$ x ($volume_{NaOH} - volume_{blank}$) = $mmol_{NaOH}$ = $mmol_{acidity}$
$mmol_{acidity}$/ $volume_{supernatant}$ = [illegible]
[illegible] x ($volume_{KCl}/mass_{sample}$) = **extractable acidity ($mmol_c$ kg $soil^{-1}$)**

Analysis and data appraisal

Extractable acidity determination was done in duplicate for some samples after approximately a week, precision is presented in Table B.7. The duplication was complete, including titration as well as extraction with KCl and, therefore, characterises two separate sub-samples. The reproducibility was acceptable with all RSD below 9%.

Table B.8 Precision of extractable acidity data.

Sample	Acidity ($mmol_c kg^{-1}$) (18/8/99)	Acidity ($mmol_c kg^{-1}$) (30/8/99)	Mean	SD	RSD(%)
ggs3	16.6	18.6	17.6	1.4	8.0
gfs3	41.3	40.6	41.0	0.5	1.2
kgs3	18.7	18.8	18.8	0.1	0.4
kfs3	113.3	112.8	113.1	0.4	0.3
kfs5	31.2	27.6	29.4	2.5	8.7

B.4.2. NH_4OAc extractable base cations

Theory

The soil sample is combined with NH_4OAc such that a mass action effect removes exchangeable cations which are replaced with NH_4^+.

Method

Extractable base cations were determined according to Grant (1982) by Infruitech in Stellenbosch. Base cations were extracted with 1 N NH_4OAc adjusted to pH 7. The concentrations of Ca^{2+}, Mg^{2+}, K^+ and Na^+ were determined in the NH_4OAc extracts with atomic absorption flame spectroscopy.

Analysis and data appraisal

The precision of the data is evaluated in Tables B.9, B.10, B.11 and B.12 for extractable Ca^{2+}, Mg^{2+}, K^+ and Na^+, respectively. The data is generally within RSD(%) < 20%, which is considered suitable. There are some exceptions, particularly with Mg, but the large errors are generally associated with low concentrations as expected.

Table B.9 Precision of NH_4OAc extractable Ca data.

Sample	Ca ($mmol_c kg^{-1}$)	Ca ($mmol_c kg^{-1}$)	Mean Ca	SD	RSD(%)
ggs3	2.32	2.42	2.37	0.07	3.0
gfs3	1.13	1.33	1.23	0.14	11.8
kgs3	4.21	3.98	4.09	0.16	3.8
kfs3	1.85	2.18	2.01	0.23	11.5

Table B.10 Precision of NH_4OAc extractable Mg data.

Sample	Mg ($mmol_c kg^{-1}$)	Mg ($mmol_c kg^{-1}$)	Mean Mg	SD	RSD(%)
ggs3	0.61	1.31	0.96	0.50	**52.1**
gfs3	0.20	0.31	0.26	0.07	**28.3**
kgs3	1.33	1.22	1.27	0.08	6.1
kfs3	4.14	3.48	3.81	0.46	12.1

Table B.11 Precision of NH_4OAc extractable Na data.

Sample	Na ($mmol_c kg^{-1}$)	Na ($mmol_c kg^{-1}$)	Mean Na	SD	RSD(%)
ggs3	0.40	0.30	0.35	0.07	20.2
gfs3	0.51	0.72	0.61	0.14	23.6
kgs3	1.00	1.00	1.00	0.00	0.0
kfs3	1.74	2.07	1.91	0.23	12.1

Table B.12 Precision of NH_4OAc extractable K data.

Sample	K ($mmol_c kg^{-1}$)	K ($mmol_c kg^{-1}$)	Mean K	SD	RSD(%)
ggs3	0.71	0.81	0.76	0.07	9.4
gfs3	0.51	0.51	0.51	0.00	0.0
kgs3	1.11	1.11	1.11	0.00	0.0
kfs3	1.20	1.09	1.14	0.08	6.7

B.5 2 M KCl-extractable NO_3 and NH_4

Soil was extracted with 2 M KCl at a soil : solution ratio of 1:10 (1:3 for anaerobic mineralisation experiment) for 30 minutes and then filtered through a Whatman #1 qualitative filter. A 2-molar solution was used primarily for two reasons, to provide sufficient ions, K^+ and Cl^-, to produce a mass action effect to remove any NH_4^+ and NO_3^- from exchange sites, and to provide a significant osmotic potential to inhibit further microbial transformations (Stock, 1983). All standards and blanks were made up with 2 M KCl and filtered through a Whatman #1 qualitative filter.

B.5.1. Copperized cadmium reduction - NO_3

Theory

Nitrate is reduced to NO_2^- in the presence of Cd granules that have been treated with $CuSO_4$. The amount of NO_2^- is determined with a modified Griess-Ilosvay method that consists of treatment of extract with a diazotizing reagent (sulphanilamide) in HCl and a coupling reagent [*N*-(1-napthyl)-ethylene] resulting in the formation of an azo chromophore. The amount of

NO_2^- is proportional to the intensity of the reddish colour produced (Keeney and Nelson, 1982).

Method

A 3 ml aliquot of soil extract was placed in a 100 ml test tube. Approximately 2 g of wet weight Cu/Cd* was added to the extract containing 0.1 ml $MgCl_2$ (in order to overcome phosphate interference) and 1.9 ml of 0.4 M NH_4Cl buffer adjusted to pH 9.6 with NH_4OH. The mixture was shaken for 10 minutes and then a 1 ml aliquot was removed to analyse for NO_2 by the Gries-Ilosvay method (Stock, 1983). To the 1 ml aliquot 1 ml of 1% (w/v) sulphnilamide in 1.5 M HCl and 1 ml of 0.02% (w/v) [*N*-(1-napthyl)-ethylene] were added and colour was allowed to develop for 10 minutes, after which absorbance was read at 540 nm (Stock, 1983). Nitrate concentrations were calculated from a calibration curve constructed from standards made up with KNO_3 and ranging from 0-2 μg NO_3–N/ml. A typical calibration curve is shown in Figure B.1.

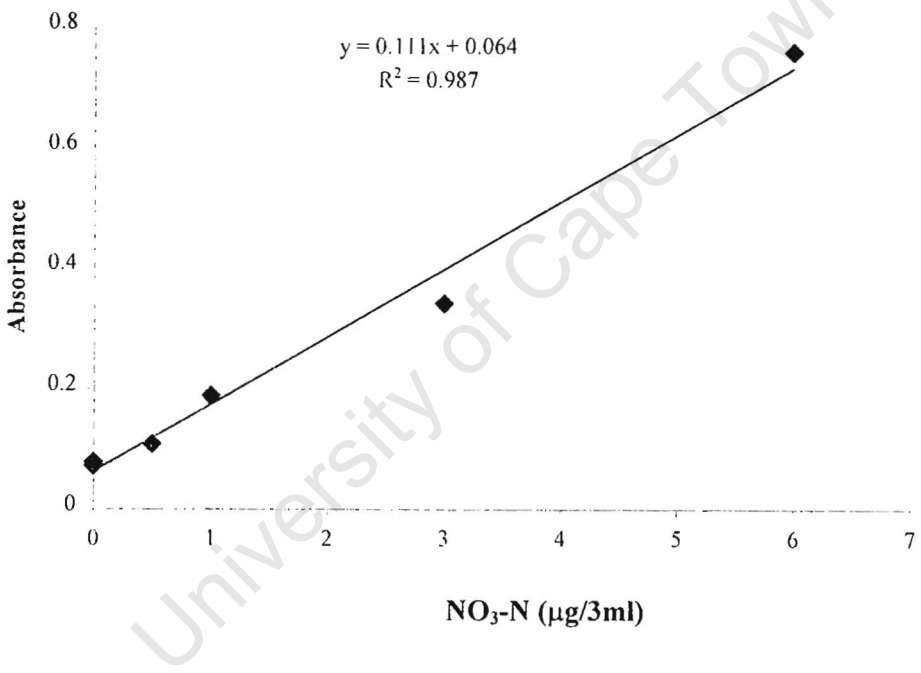

Figure B.1 Typical calibration curve for NO_3 determination by copperized cadmium reduction method

*The reduced Cu/Cd was prepared by washing coarse granules of Cd briefly with 5% HCl followed by H_2O. The Cd was then stirred with 0.5% (w/v) $CuSO_4 \cdot 5H_2O$ and filtered. The Cu/Cd was then washed repeatedly with a solution containing 0.007 M HCl and 0.005 M EDTA- Na_2 to remove precipitated Cu and CuO until the supernatant was clear and the Cu/Cd silvery-grey (Bate and Heelas, 1975).

Analysis and data precision

The precision of the data is presented in Table B.13. All of the samples were reproducible within 20% and with the exception of 3 samples (ggs3, gfs3, and kfs5), the samples are reproducible within 15%. These results were acceptable for this study.

Table B.13 Precision of extractable NO_3 data.

Sample	Absorbance	Absorbance	Mean	SD	RSD(%)
ggs1	0.209	0.214	0.212	0.00	1.7
ggs2	0.205	0.209	0.207	0.00	1.4
ggs3	0.175	0.221	0.198	0.03	16.4
ggs4	0.192	0.203	0.198	0.01	3.9
ggs5	0.181	0.200	0.191	0.01	7.1
gfs1	0.254	0.253	0.254	0.00	0.3
gfs2	0.270	0.239	0.255	0.02	8.6
gfs3	0.274	0.216	0.245	0.04	16.7
gfs4	0.342	0.293	0.318	0.03	10.9
gfs5	0.204	0.185	0.195	0.01	6.9
kgs1	0.184	0.146	0.165	0.03	16.3
kgs2	0.192	0.194	0.193	0.00	0.7
kgs3	0.452	0.456	0.454	0.00	0.6
kgs4	0.363	0.295	0.329	0.05	14.6
kgs5	0.395	0.363	0.379	0.02	6.0
kfs1	0.341	0.412	0.377	0.05	13.3
kfs2	0.227	0.198	0.213	0.02	9.6
kfs3	0.206	0.194	0.200	0.01	4.2
kfs4	0.319	0.258	0.289	0.04	15.0
kfs5	0.170	0.214	0.192	0.03	16.2

B.5.2. Indophenol blue - NH_4

Theory

Phenol reacts with NH_3 in the presence of an oxidising agent (hypochlorite) under alkaline conditions to form a blue coloured complex. The intensity of the coloured complex is proportional to the concentration of NH_4 (Keeney and Nelson, 1982).

Method

A 2 ml aliquot of soil extract is placed in a test tube. Sequentially the following reagents were added: 1.6 ml of 10% (w/v) sodium potassium tartrate; 0.2 ml of 0.16% (w/v) sodium nitroprusside serving as a catalyst; 0.4 ml of sodium phenate reagent prepared daily with 25 g NaOH and 12.5 g phenol made up to 100 ml with deionized H_2O; and 0.2 ml of sodium hypochlorite with 5% available Cl^-. The tubes were made up to 10 ml and incubated at 40°C in a water bath for 20 minutes after which they were cooled in an ice bath. The absorbance was read within 15 minutes at 625 nm (Stock, 1983). Ammonium concentrations were determined from a calibration curve consisting of $(NH_4)_2SO_4$ standards ranging from 0-3.5 µg NH_4-N/ml. A typical calibration curve is shown in Figure B.2

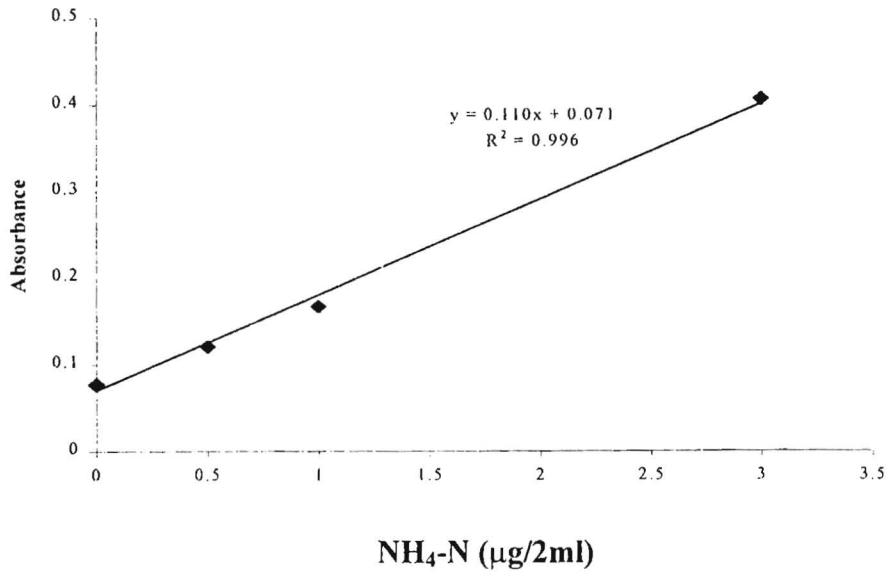

Figure B.2 Typical indo-phenol blue NH_4 determination calibration curve

Analysis and data appraisal

The extractable NH_4 determination was done in duplicate (extraction and colour development), the precision of the data is presented in Table B.14. Similar to the extractable NO_3 data, the extractable NH_4 data was reproducible to an acceptable extent, with all except one sample (ggs3) with RSD < 10%.

Table B.14 Precision of 2 M KCl-extractable NH_4 data.

Sample	Absorbance	Absorbance	Mean	SD	RSD(%)
ggs1	0.119	0.109	0.114	0.01	6.2
ggs2	0.193	0.194	0.194	0.00	0.4
ggs3	0.127	0.160	0.144	0.02	16.3
ggs4	0.093	0.101	0.097	0.01	5.8
ggs5	0.124	0.125	0.125	0.00	0.6
gfs1	0.136	0.128	0.132	0.01	4.3
gfs2	0.136	0.125	0.131	0.01	6.0
gfs3	0.148	0.149	0.149	0.00	0.5
gfs4	0.346	0.351	0.349	0.00	1.0
gfs5	0.104	0.114	0.109	0.01	6.5
kgs1	0.125	0.128	0.127	0.00	1.7
kgs2	0.153	0.154	0.154	0.00	0.5
kgs3	0.143	0.149	0.146	0.00	2.9
kgs4	0.115	0.133	0.124	0.01	10.3
kgs5	0.158	0.163	0.161	0.00	2.2
kfs1	0.176	0.177	0.177	0.00	0.4
kfs2	0.272	0.276	0.274	0.00	1.0
kfs3	0.236	0.227	0.232	0.01	2.7
kfs4	0.296	0.309	0.303	0.01	3.0
kfs5	0.19	0.215	0.203	0.02	8.7

B.6 Organic carbon determination - Walkley-Black method

Theory

Organic carbon was determined according to the Walkley-Black method (Nelson and Sommers, 1982) by Infruitech in Stellenbosch, repeat analyses were done at the University of Cape Town. The method consists of the rapid oxidation of oxidisable C followed by a back titration to determine the excess oxidising agent and by difference, the oxidisable C is determined. Since the soils are non-calcareous, oxidisable C was interpreted as organic C (Nelson, and Sommers, 1982).

Method

One g of air-dried, swing-milled soil sample was transferred to a 500 ml Erlenmeyer flask. The sample was mixed with 10 ml $K_2Cr_2O_7$ solution. 20 ml of concentrated H_2SO_4 was added to the solution and allowed to cool for 30 minutes. Then 150 ml distilled water, 10 ml concentrated ortho-phosphoric acid, and 1-ml indicator were added. The excess dichromate was titrated with $Fe(NH_4)_2(SO_4)_2$ solution to the green endpoint.

The concentration of the $Fe(NH_4)_2(SO_4)_2$ solution was determined by titrating a blank of 10 ml of 0.167 M $K_2Cr_2O_7$ solution. The calculation is as follows:

$$\text{molar concentration of } Fe(NH_4)_2(SO_4)_2 = \frac{10 \text{ ml } K_2Cr_2O_7 \text{ x } 0.167 \text{ M x } 6}{\text{ml } Fe(NH_4)_2(SO_4)_2}$$

The percent organic carbon was determined by titrating a mixture of sample and $K_2Cr_2O_7$ solution mixture with the standardised $Fe(NH_4)_2(SO_4)_2$ solution. The calculation is as follows:

$$\text{organic carbon \%} = \frac{[\text{ml } Fe(NH_4)_2(SO_4)_2\text{blank} - Fe(NH_4)_2(SO_4)_2\text{sample}]\text{x M x } 0.3 \text{ x f}}{\text{soil mass (g)}}$$

where M = concentration $Fe(NH_4)_2(SO_4)_2$ and f = a recovery factor of 1.3.

Analysis and data appraisal

The reproducibility of the data is presented in Table B.15. All results are within 15% RSD, indicating acceptable reproducibility.

Table B.15 Precision of organic carbon analyses.

Sample	OC (%)	OC (%)	OC (%)	OC (%)	Mean	SD	RSD(%)
ggs1	10.9	10.7	10.8	9.9	10.6	0.43	4.1
ggs2	3.0	2.7			2.8	0.24	8.5
ggs3	3.0	2.7			2.8	0.18	6.3
ggs4	0.8	1.0	1.1	1.1	1.0	0.15	15.1
ggs5	3.5	3.5			3.5	0.04	1.2
gfs1	9.0	8.7	9.0	10.1	9.2	0.62	6.7
gfs2	8.7	8.5	8.4	8.4	8.6	0.14	1.6
gfs3	4.4	4.6			4.5	0.11	2.5
gfs4	9.4	10.1			9.8	0.53	5.4

Table B.15 Precision of organic carbon analyses (continued).

Sample	OC (%)	OC (%)	OC (%)	OC (%)	Mean	SD	RSD(%)
gfs5	3.6	3.3			3.4	0.23	6.6
kgs1	3.3	3.3			3.3	0.01	0.2
kgs2	5.3	5.3			5.3	0.00	0.0
kgs3	9.7	9.7			9.7	0.04	0.4
kgs4	1.6	1.3			1.5	0.16	11.3
kgs5	6.6	6.7			6.6	0.06	0.9
kfs1	3.8	4.1			3.9	0.21	5.3
kfs2	6.4	6.2			6.3	0.13	2.0
kfs3	8.1	8.3			8.2	0.12	1.5
kfs4	6.9	6.8	6.3	6.4	6.6	0.33	4.9
kfs5	4.6	4.3	4.8	4.0	4.4	0.37	8.3

B.7 Total nitrogen

Theory

Total N was determined by the Kjeldahl digestion technique (salicylic acid-thiosulphate modified to include nitrate and nitrite) according to Bremner and Mulvaney (1982). The method consists of a digestion, converting organic N to NH_4^+-N, followed by quantitative colorimetric determination of NH_4^+-N with a modified indo-phenol blue technique.

Method

One g aliquots of soil were placed in a long, thick-walled boiling tube. One ml of distilled H_2O was added, followed by 3 ml of acidified salicylic acid, approximately 1 g of sodium thiosulphate crystals and 1 Se Kjeldahl tablet. Tubes were placed in a cold digestion block and heated to 150°C for 2 hours, 250°C for 1 hour, 300°C for half an hour and 350°C for 3 hours. The heating was repeated at half-hour increments until the digest went clear. Each digest was made up to 50 ml with distilled H_2O.

The colour was developed with 0.5 ml of digest, 25 ml of 0.12% (w/v) EDTA-Na_2, 2 ml of reagent A (equal volumes of 0.5% (w/v) sodium nitroprusside and 10% phenol in 95% ethanol) and 3.5 ml of reagent B (4 parts alkaline phosphate buffer and 1 part 1.5% sodium hypochlorite). The tubes were mixed thoroughly and made up to 50 ml with distilled H_2O. Colour developed within an hour and absorbance was read at 635 nm. The concentration of N was determined from a calibration curve consisting of $(NH_4)_2SO_4$ standards ranging between 0-4 mg-N. A calibration curve is shown in Figure B.3.

Analysis and data appraisal

The precision of the analysis is presented in Table B.16. The analyses were reproducible within 30%, with the exception of one sample and considered suitable for the purposes of this study.

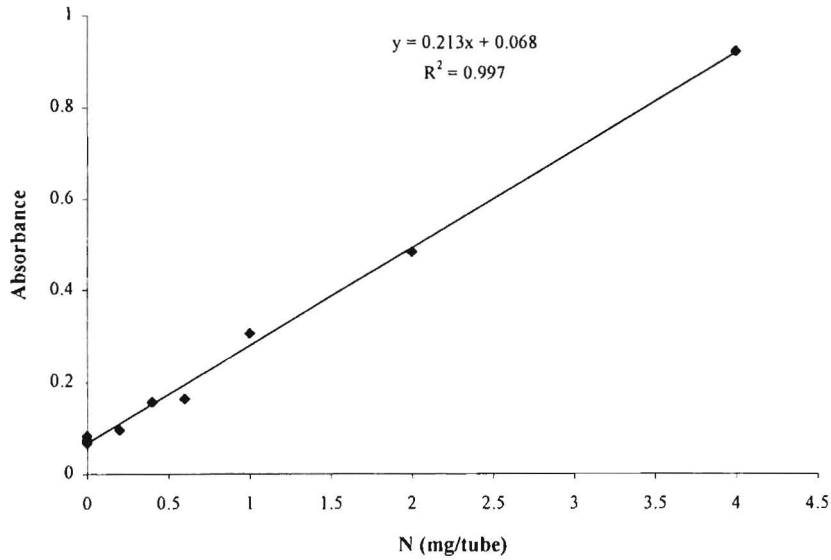

Figure B.3 Total N calibration curve

Table B.16 Precision of total N data

Sample	Total N (%)	Total N (%)	Mean	SD	RSD(%)
ggs1	0.45	0.35	0.40	0.07	17.7
ggs4	0.05	0.04	0.05	0.01	15.7
gfs2	0.33	0.32	0.33	0.01	2.2
kfs5	0.12	0.18	0.15	0.04	28.3

B.8 Texture - particle size analysis

Particle size analysis was determined according to the hydrometer method (Gee and Bauder, 1982) at Infruitech, Stellenbosch.

Analysis and data appraisal

The precision of the particle size analysis is presented in Table B.17, which shows acceptable reproducibility considering separate sub-samples were used for duplicate analysis.

Table B.17 Precision of particle size analysis.

Sample	ggs1	gfs2	kfs5
Clay	4.4	2.8	18.2
Clay	3.6	5.6	16.4
Mean	4.0	4.2	17.3
SD	0.6	2.0	1.3
RSD (%)	14.1	**47.1**	7.4

Table B.17 Precision of particle size analysis (continued).

Sample	ggs1	gfs2	kfs5
Silt	9.6	13.4	22.0
Silt	12.8	16.1	22.6
Mean	11.2	14.8	22.3
SD	2.3	1.9	0.4
RSD (%)	**20.2**	12.9	1.9
Fine sand	74.8	64.2	35.8
Fine sand	75.6	63.7	39.8
Mean	75.2	64.0	37.8
SD	0.6	0.4	2.8
RSD (%)	0.8	0.6	7.5
Medium sand	3.6	10.8	8.8
Medium sand	2.8	9.6	8.0
Mean	3.2	10.2	8.4
SD	0.6	0.8	0.6
RSD (%)	17.7	8.3	6.7
Coarse sand	7.6	8.8	15.2
Coarse sand	5.2	5.0	13.2
Mean	6.4	6.9	14.2
SD	1.7	2.7	1.4
RSD (%)	**26.5**	**38.9**	10.0

B.9 Sorption experiment

A sorption experiment was conducted to investigate the NO_3^- sorption capacity of the soil samples. Four samples (ggs2, gfs3, kgs4 and kfs3) characterised by high acidity and low pH, consequently expected to contain the largest AEC, were chosen for the experiment. Five treatments were used: 0 0, 3.4, 6.2, 10.7, and 14.2 mmol KNO_3 kg^{-1}. Five grams of sample was equilibrated with 25 ml solution (containing the treatment amounts of KNO_3) for 30 minutes on a shaker. The supernatant of the soil-solution mixture was filtered through a 0.2 μm filter, all samples, except for the 0 treatment, were then diluted 10 times. Samples were analysed for major anions with IC (section B.3.2).

B.10 Soil to extract ratio dilution experiment

A dilution experiment was conducted in order to assess the effect of varying soil : extract ratios on the amount of extractable NO_3^-. Deionized water was used for extraction and soil : water ratios were 1:1, 1:2, 1:5, and 1:10. The experiment was conducted with an air-dried sample (ggs2) and a field-moist sample (gfs3) in order to assess the effect of air-drying on NO_3^- concentrations in soil extracts.

B.11 Incubation experiments

An appraisal of experimental methodology and reproducibility of the data from the incubation experiments is presented in Chapter 3.

B.11.1. Aerobic

Theory

An aerobic N mineralisation experiment was conducted with an NH_4^+-N amendment in order to determine the nitrifying potential of the soils. In untreated soil the rate of NO_3^- formation is generally limited by the rate at which NH_4^+ is formed from ammonification of organic N. The addition of NH_4^+-N ensures that the soil will be non-limiting with respect to nitrifiable substrate and the nitrifying population is expected to increase until limited by some other factor or combination of factors. The experiment is limited in its application due to the absence of vegetation and of temperature and moisture fluctuations. It is, however, useful as a means to compare different soils with different properties (Schmidt and Belser, 1982).

Method

A nitrifying potential aerobic mineralisation experiment was conducted according to Schmidt and Belser (1982). Field moist sample that had been refrigerated was used for the experiment. An oven-dried equivalent mass of 25 g of sample was treated with 250 μmol_c of NH_4^+ in the form of $(NH_4)_2SO_4$ in solution. Water was added until field capacity was reached (field capacity was taken to be half of saturation which had been determined by making saturated pastes - section B.3). The experiment was run in duplicate and relative to control samples, which had not been treated with NH_4^+. The duplicate samples and controls were incubated at ~25-30°C for 28 days. The NO_3^- at time 0, 14 and 28 days was extracted with 2 M KCl, filtered through a Whatman #1 qualitative filter and analysed using the copperized cadmium method (Stock, 1983; section B.5.1).

B.11.2. Anaerobic

Theory

An anaerobic N mineralisation experiment was conducted under waterlogged conditions in sealed vessels according to Keeney (1982). In the waterlogged incubation, biological activity is expected to maintain anaerobic conditions, eliminating nitrification-denitrification reactions at the soil-water interface. Since ammonification is expected to be rate limiting in NO_3^- formation, the anaerobic method provides a suitable index of N availability. Advantages of the anaerobic method compared to the aerobic method include standardised conditions with respect to water content and the use of higher temperatures (40°C) (which ensures rapid mineralisation) since optimum temperature is not as crucial as it would be in the case of nitrification (Keeney, 1982). As with the aerobic incubation, however, the method is limited in application due to the absence of vegetation and of temperature and moisture fluctuations, but it does provide a means of useful comparison between soils.

Method

For the anaerobic experiment, an oven-dried equivalent mass of 5 g of sample was waterlogged with 15 ml of deionized H_2O. The samples were sealed and incubated at 40°C for 7 days. The experiment was run in duplicate and relative to controls that were kept frozen at 0°C for the same time period. The water-logged samples were extracted with 15 ml of 2 M KCl after 7 days, filtered through a Whatman #1 qualitative filter and analysed for NH_4 using the indo-phenol blue method (Keeney and Nelson, 1982).

Appendix C. Cluster analysis and dilution experiment discussion

C. 1 Cluster analysis: samples excluded from assessment of afforestation-induced chemical changes

The cluster analysis discussed in Chapter 2 identified thirteen samples (six grassland and seven forest) for which vegetation is a legitimate grouping variable. The remaining seven samples were excluded from interpretation of the impacts of afforestation on soil chemical properties. A discussion of the seven excluded samples speculating possible reasons for exclusion follows.

Sample ggs1: This soil is characterised by high organic matter content (organic carbon = 10.6%) and consequently high total nitrogen content (0.40%) attributed to poor drainage. Field observations characterised the soil as very moist, dark, rich in humus and almost peaty in character. The enhanced organic carbon and total nitrogen in this sample exceeded the median values for the forest samples (6.3% and 0.33%, for organic carbon and total nitrogen, respectively), and the sample was, consequently clustered with the forest samples.

Sample kgs2: This sample is characterised by relatively low EC (189 μScm^{-1}), which is lower than the median for the forest samples. Likewise, the sample is characterised by intermediate organic carbon content (5.3%) and total nitrogen (0.29%) more similar to the forest samples than the grassland samples. Furthermore, the underlying geology for this soil is granite unlike most other soils in the study, perhaps contributing the fact that texturally it is more similar to the forest soils than the grassland soils. As with sample ggs1, sample kgs2 was clustered with the forest samples.

Sample kgs3: This soil is characterised by a high organic carbon content (9.7%) and total nitrogen (0.58%) which is higher than the median forest values for both parameters. The elevated total nitrogen is also apparent in an elevated inorganic nitrogen status, for example, extractable NO_3^- is 0.70 mmol kg^{-1}, higher than any other soil sampled, irrespective of vegetation. Sample kgs3 was also clustered with the forest samples.

Sample kgs5: This soil is characterised by relatively high clay content (6.2%), reflecting the underlying diabase geology, high organic carbon (6.6%) and high total nitrogen (0.41%). Sample kgs5 was consequently also clustered with the forest samples.

Sample kfs3: This soil is in a 40 year old *Pinus patula* stand and is characterised by a substantial fermented litter layer. The soil is extremely acidic (pH = 3.26; extractable-acidity = 123.1 $mmol_c kg^{-1}$) and was, therefore, not clustered with the forest samples nor with the grassland samples.

Sample kfs4: This soil was more acidic than the median forest sample (pH = 4.49; extractable-acidity = 43.8 $mmol_c kg^{-1}$). It is also characterised with an extremely high C/N ratio (41), with an organic carbon content of 6.6% and

extremely low total nitrogen content of 0.16%. Similarly to sample kfs3, sample kfs4 was not clustered with the forest or with the grassland samples.

Sample kfs5: This soil was also not clustered with the grassland or with the forest samples. Texturally the soil is characterised by a clay content of 17.3%, higher than any other soil sampled regardless of vegetation, reflecting perhaps, different parent material than the majority of soils sampled. The underlying geology is arkose conglomerate.

C. 2 Dilution experiment

The soil to extract dilution experiment described in Chapter 3 was considered inconclusive due to spurious concentrations of SO_4^{2-}, Cl^-, Na^+ and NH_4^+ suggesting the possibility of contamination. The following four Figures (C.1, C.2, C.3, C.4) present the concentrations of the cations and anions for the dilution series or both samples.

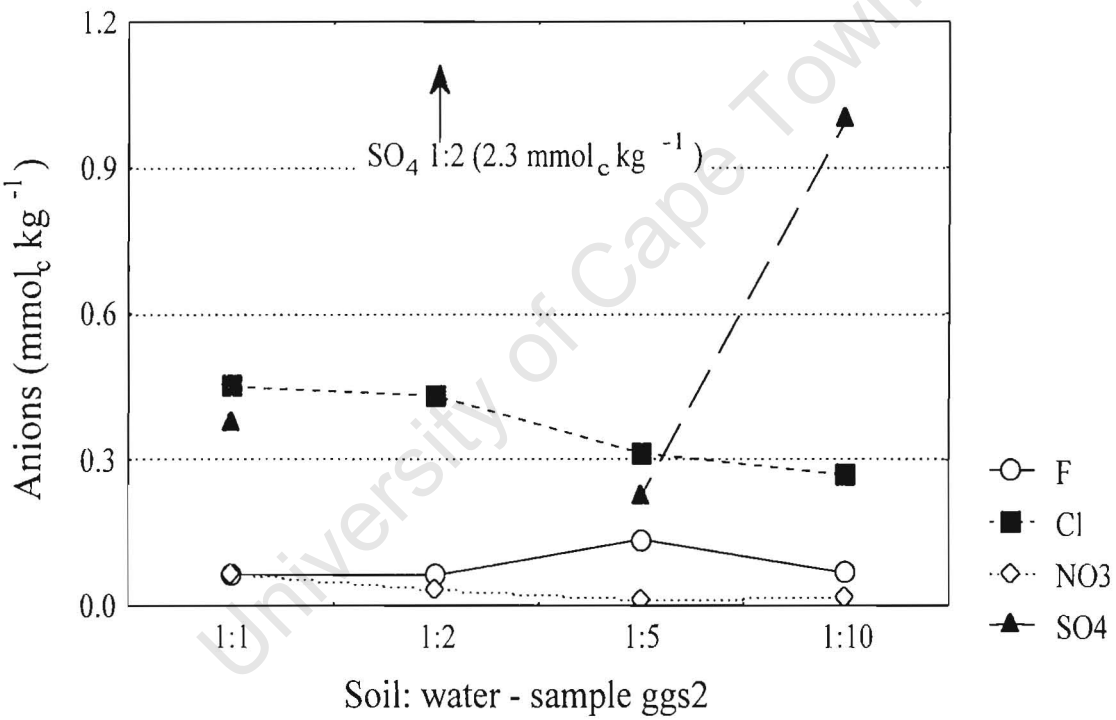

Figure C.1 Results of dilution experiment: anions for sample ggs2

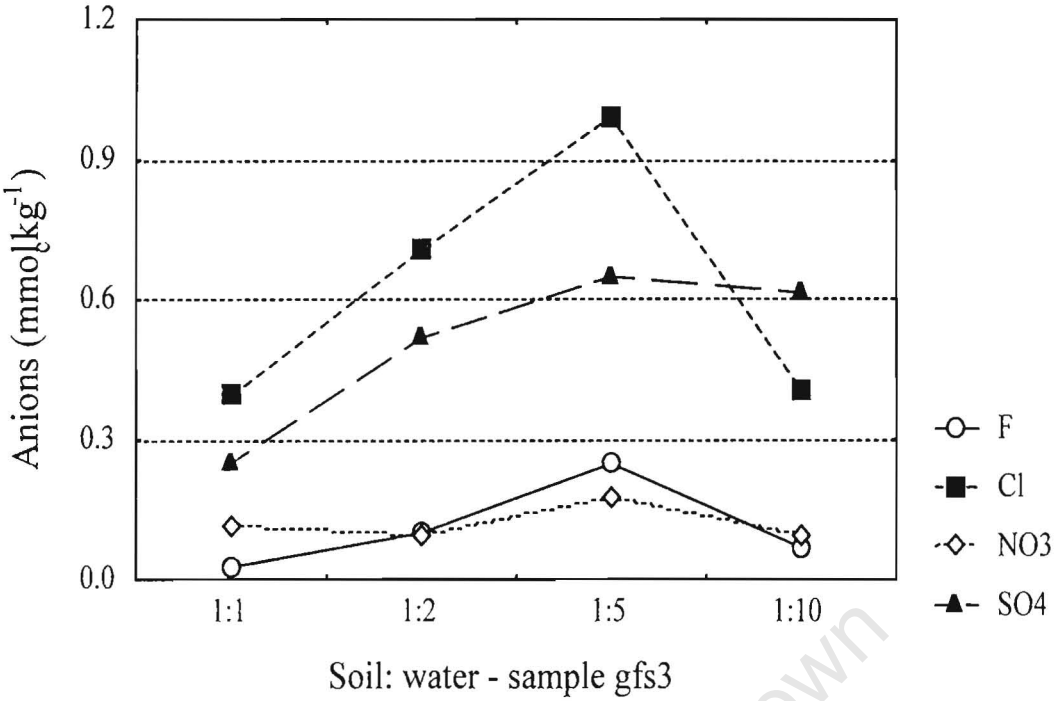

Figure C.2 Results of dilution experiment: anions for sample gfs3

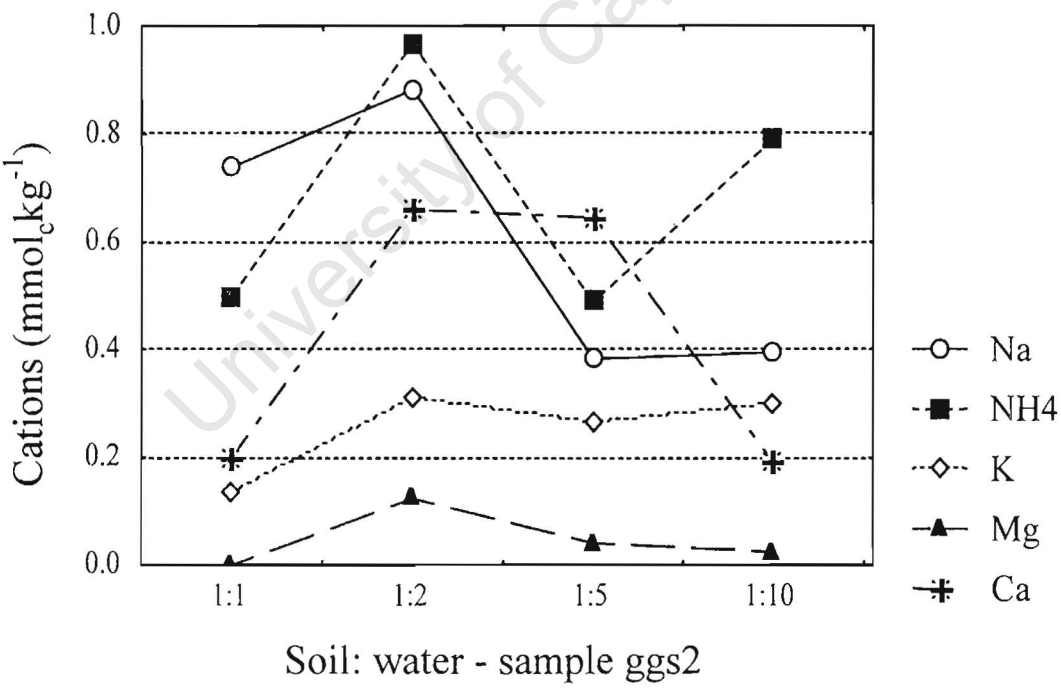

Figure C.3 Results of dilution experiment: cations for sample ggs2

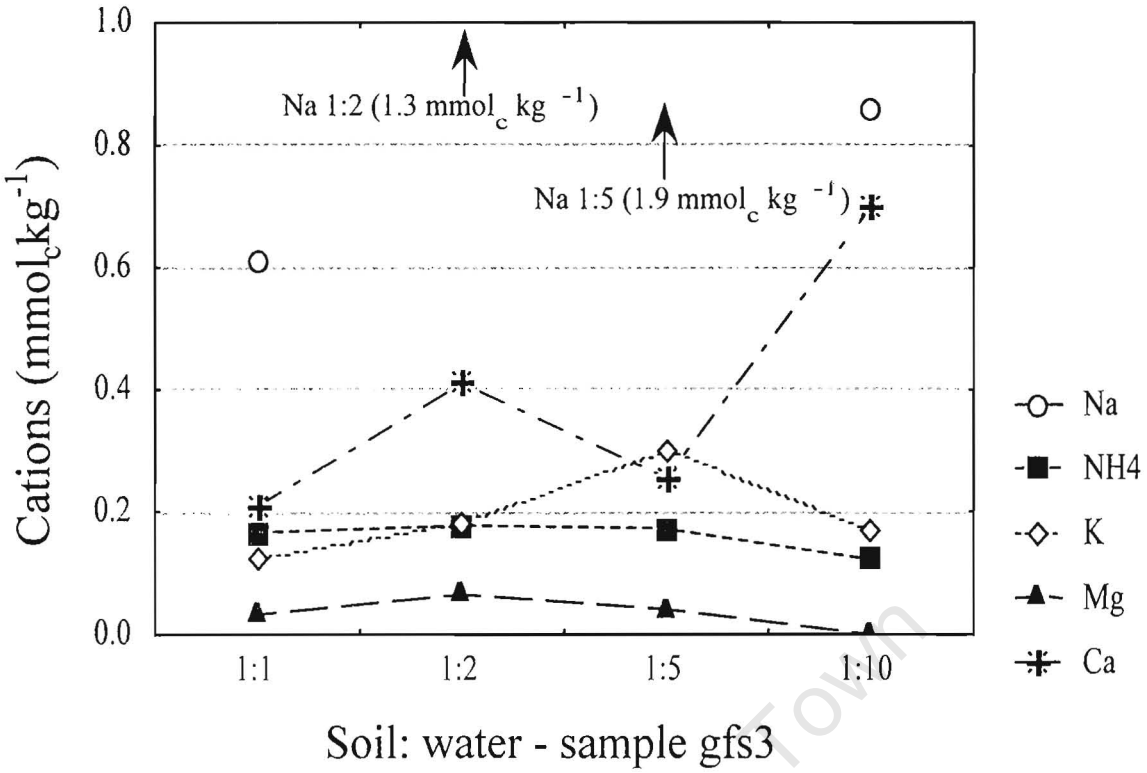

Figure C.4 Results of dilution experiment: cations for sample gfs3